ELUCIDATION OF THE ANTIOXIDANT STATUS IN RESPONSE TO NON SURGICAL PERIODONTAL THERAPY AND POST VITAMIN E SUPPLEMENTATION IN PATIENTS WITH CHRONIC PERIODONTITIS-A RANDOMISED CONTROLLED TRIAL

Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

In partial fulfilment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH II
DEPARTMENT OF PERIODONTICS
MAY 2018

CERTIFICATE

This is to certify that this dissertation titled, **"ELUCIDATION OF THE ANTIOXIDANT STATUS IN RESPONSE TO NON SURGICAL PERIODONTAL THERAPY AND POST VITAMIN E SUPPLEMENTATION IN PATIENTS WITH CHRONIC PERIODONTITIS-A RANDOMISED CONTROLLED TRIAL"** is a bonafide record of work done by **Dr. MEGHA MADHUSOODANAN** under our guidance and to our satisfaction, during her postgraduate study period of 2015-2018.

This dissertation is submitted to **THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY** in partial fulfilment for the award of the degree of **MASTER OF DENTAL SURGERY - PERIODONTICS, BRANCH II**. It has not been submitted (partial or full) for the award of any other degree or diploma.

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TITLE OF DISSERTATION	**ELUCIDATION OF THE ANTIOXIDANT STATUS IN RESPONSE TO NON SURGICAL PERIODONTAL THERAPY AND POST VITAMIN E SUPPLEMENTATION IN PATIENTS WITH CHRONIC PERIODONTITIS-A RANDOMISED CONTROLLED TRIAL**
PLACE OF STUDY	**SRI RAMAKRISHNA DENTAL COLLEGE AND HOSPITAL, COIMBATORE-641006.**
DURATION OF THE COURSE	**3 YEARS**
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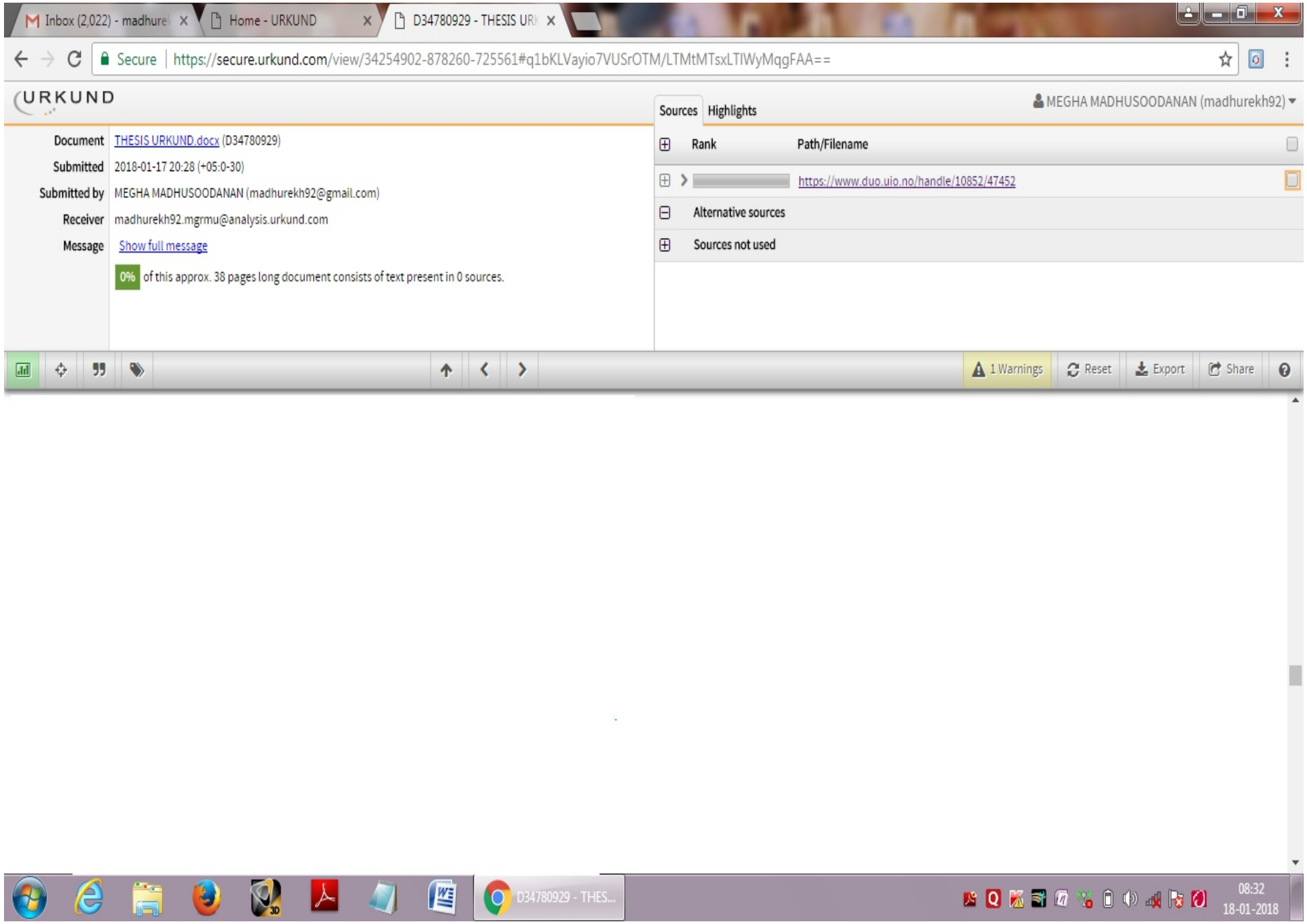
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"A good teacher is a doctor who heals the ignorance and an artist who inspires creativity"

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"God incarnate is the end of fear"

Finally**,** I am grateful to **The Almighty God**, for guiding me in every day of my life.

- **MEGHA MADHUSOODANAN**

ABSTRACT

BACKGROUND:

Chronic Periodontitis is an infectious disease resulting in inflammation within the supporting tissues of the teeth leading to progressive attachment loss and bone loss. It is characterized by the liberation of reactive oxygen species, that has deleterious effects on these tissues and is eliminated by various protective antioxidant mechanisms. Superoxide dismutase (SOD) is known to be one of the most prominent antioxidant enzymes in the body and its levels are considered to be of clinical significance in detecting the varying degrees of tissue destruction. Vitamin E is a group of naturally occurring tocopherols that possesses antioxidant properties. Therefore it will be informative to evaluate the cumulative effect of Chronic Periodontitis with and without Vitamin E supplementation on the levels of SOD in the gingival crevicular fluid (GCF).

AIM:

To elucidate the levels of Superoxide Dismutase in response to Non Surgical Periodontal Therapy with and without Vitamin E supplementation in patients with Chronic Periodontitis.

MATERIALS AND METHODS:

This study was performed on 46 participants which included 17 patients with chronic periodontitis supplemented without Vitamin E (Group II), 17 patients with chronic periodontitis supplemented with Vitamin E (Group III) and 12 systemically healthy individuals with clinically healthy periodontium who served as controls (Group I). The clinical parameters [Plaque Index (PI), Probing Depth (PD), Clinical Attachment Level (CAL), Modified Sulcus bleeding index (mSBI%)] and biochemical parameters

like the GCF- SOD levels were measured at baseline. All the patients except healthy controls were treated with scaling and root planing followed by evaluation of the above mentioned clinical and biochemical parameters after 6 weeks.

RESULTS:

Results showed that all the clinical parameters improved after scaling and root planing in Group II and Group III, with the patients in Group III showing a greater reduction in PI ($p<0.05$), PD ($p<0.05$), mSBI% ($p<0.05$) and greater gain in clinical attachment level ($p<0.05$) when compared to Group II patients and healthy controls and, this difference was statistically significant. With respect to the levels of Superoxide dismutase in gingival crevicular fluid, patients in Group III showed a greater reduction, 6 weeks post scaling and root planing when compared to Group II patients and this difference was statistically significant ($p<0.05$).

CONCLUSION:

From the results obtained, it was concluded that scaling and root planing reduces Superoxide dismutase levels in the gingival crevicular fluid of patients with chronic periodontitis with significant reduction appreciated in Group III patients. The group being subjected to Vitamin E supplementation exhibited better improvement clinically and biochemically, 6 weeks post scaling and root planing.

KEY WORDS:

Chronic Periodontitis, reactive oxygen species, antioxidant, superoxide dismutase, vitamin, tocopherols, vitamin E supplementation, Gingival Crevicular Fluid.

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LIST OF ABBREVIATIONS

ROS	:	Reactive oxygen species
RNS	:	Reactive nitrogen species
SOD	:	Superoxide dismutase
LPS	:	Lipopolysaccharide
GCF	:	Gingival Crevicular Fluid
FR	:	Free Radicals
ODFR	:	Oxygen derived free radicals
ATP	:	Adenosine triphosphate
AChE		Acetylcholinesterase
RNA	:	Ribo Nucleic Acid
DNA	:	Deoxyribo Nucleic Acid
PMN	:	Polymorphonuclear neutrophils
CL	:	Chemi-luminescence
s.d	.:	Standard deviation
CAP	:	Chronic adult periodontitis
CP	:	Chronic Periodontitis
HCl	:	Hydrochloride
MT	:	Metallotheoin
OR	:	Odds ratio
CAT	:	Catalase
TAOC	:	Total antioxidant capacity
MDA	:	Malondialdehyde
NSB	:	Non-specific binding antigen
TOS	:	Total oxidant status
AO	:	Antioxidant

PMCP	:	Post-menopausal patients with chronic periodontitis
PCP	:	Pregnant patients with CP
PCP1	:	Pregnant patients with CP in the first trimester
PG	:	Pregnant patients with gingivitis
PG1	:	Pregnant patients with gingivitis in the first trimester
GBI	:	Gingival bleeding index
BOP%	:	Bleeding On Probing
mSBI	:	Modified sulcus bleeding index
CAL	:	Clinical Attachment Level
AL	:	Attachment loss
CP-D	:	Chronic Periodontitis with Type II Diabetes Mellitus
CRP	:	C-reactive Protein
GPx	:	glutathione peroxidise
GI	:	Gingival Index
PD	:	Probing Depth
PI	:	Plaque Index
CEJ	:	Cemento-enamel junction
SRP	:	Scaling and root planing
ELISA	:	Enzyme Linked Immunosorbent Assay
TAOC	:	Total antioxidant capacity

INTRODUCTION

INTRODUCTION

Chronic Periodontitis is an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss and bone loss.[1] Periodontitis is primarily caused by the presence of microbial plaque in conjugation with exaggerated local factors and systemic host response that may influence the overall health and status of the patient.[2] Periodontitis is propagated by various pathogenic mechanisms amongst which the liberation of reactive oxygen species (ROS) has proved to cause extensive tissue breakdown. Normally, the generation of these molecules is eliminated by various antioxidant mechanisms present in the human body.[3]

Antioxidants help in scavenging free radicals generated in the body. Antioxidants may be defined as ***"A substance that significantly decreases the adverse effects of reactive species such as Reactive oxygen species (ROS) or Reactive nitrogen species (RNS) on normal physiological function in humans"*** [4]

Some of the antioxidants produced in the human body include, superoxide dismutase, uric acid, ascorbic acid, glutathione, peroxyl, perhdroxyl, alkoxyl, arylperoxyl, acyloxyl, acylperoxyl amongst which superoxide dismutase (SOD) is an enzyme that simultaneously catalizes the dismutation of the superoxide radical into either molecular oxygen or hydrogen peroxide. Superoxide dismutase (SOD) is the most prominent intracellular antioxidant enzyme.[5] produced as an important by product of oxygen metabolism.

Gingival crevicular fluid (GCF) is a serum derived exudate that originates from periodontal connective tissues and exits via the gingival sulcus into the oral cavity. Various enzymatic and non-enzymatic biomarkers of both host and bacterial

origin are present in GCF which makes it a potential diagnostic tool in assessing the biological state of the periodontium in both health and disease.[6]

Vitamins are a group of organic nutrients, required in small quantities for performing various biochemical functions, that usually cannot be synthesized by the human body and therefore need to be supplemented in the diet.[7] Amongst the class of Vitamin s, Vitamin -E is a fat soluble Vitamin and this class of Vitamin , like other antioxidants can scavenge free radicals and prevent oxidative damage to lipid membranes.

Vitamin E is composed of a family of 8 related compounds, the tocopherols and the tocotrienols. The major chemical forms of Vitamin –E are the tocopherols which is classified based on the location of a methyl (CH_3) group. It is divided into 4 sub-groups: Alpha, Beta, Delta and Gama. Alpha-tocopherol is the most abundant form of Vitamin E present in foods and is generally used in the form of supplements.

Keeping in mind with this background, the aim of the study is to elucidate the levels of Superoxide dismutase (SOD) in GCF in response to non surgical periodontal therapy with and without Vitamin E supplementation in patients with Chronic Periodontitis in conjugation with the assessement of clinical parameters.

AIM & OBJECTIVES

AIM:

To elucidate the levels of Superoxide Dismutase in response to non surgical periodontal therapy with and without Vitamin E supplementation in patients with chronic periodontitis.

OBJECTIVES:

1. To assess and compare the levels of Superoxide dismutase in the gingival crevicular fluid of healthy and chronic periodontitis patients.
2. To assess and compare the levels of Superoxide dismutase in the gingival crevicular fluid of chronic periodontitis patients with and without Vitamin E supplementation at baseline and six weeks post scaling and root planing.
3. To determine the clinical changes in patients with chronic periodontitis with and without Vitamin E supplementation at baseline and 6 weeks following scaling and root planing.

REVIEW OF LITERATURE

LIBERATION OF REACTIVE OXYGEN SPECIES IN PATHOGENIC MECHANISMS

HISTORY AND TERMINOLOGY

A free radical (FR) can be defined as any species capable of independent existence that contains one or more unpaired electrons, an unpaired electron being one that is alone in an orbital.[9]

Antioxidant is defined as any substance that, when present at low concentrations compared with that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate.[10]

Oxidative stress is defined as an imbalance between oxidants and antioxidants in favour of the oxidants, potentially leading to damage.[11]

Organism welfare depends on the activity of efficient defence systems against oxidative damage induced by free radicals or reactive oxygen species. The discovery of SOD in 1967 lead to the initiation of an effective antioxidant enzyme system in the human body. [12]

Brewer recognized this protein while performing the protein analysis of starch gels using the phenazine-tetrazolium technique, which he named as an indophenol oxidase, that was later coined as superoxide dismutase by Irwin Fridovich and Joe M. McCord at Duke University in 1968.[13,14]

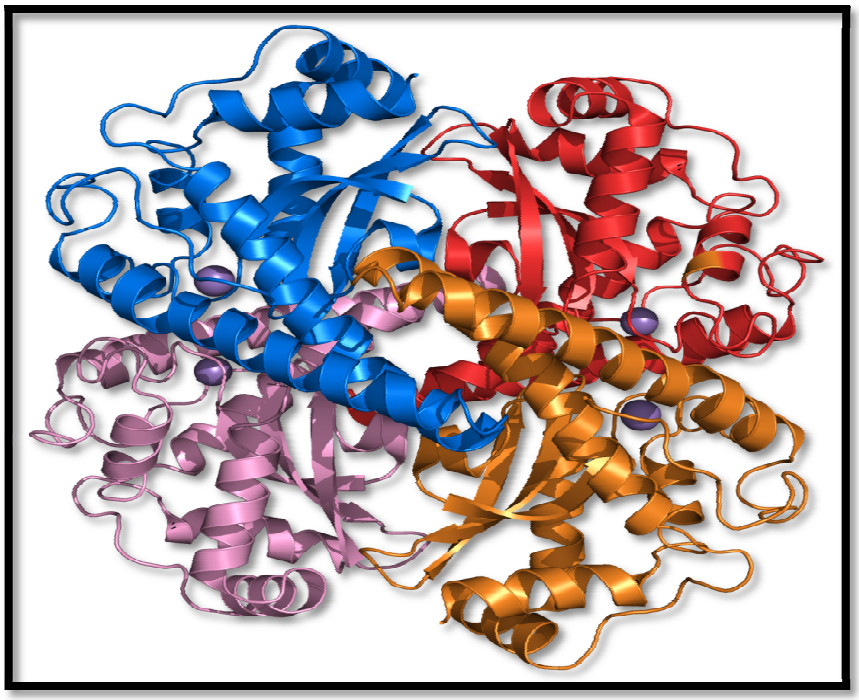


Fig. 1: Human Superoxide Dismutase Tetrameric Structure[15]

GENESIS OF REACTIVE OXYGEN SPECIES (ROS)

Oxygen was discovered by the Swedish scientist Carl Wilhelm Scheele and he recorded in his thesis ***Luft und dem Feuer*** (Air and Fire) from Uppsala and Leipzig in 1777.[16,17] Molecular oxygen (O_2) is in a triplet ground state.

It contains even number of electrons, two of them unpaired in its molecular orbitals[18]

They have the same spin quantum number, i.e., they display parallel spins. When O_2 oxidizes another atom (or molecule) by receiving a couple of electrons from it, both of them should be of parallel spin to fit into the space available in the orbitals.

Each of the finally complete orbitals and has a pair of electrons with regard to their antiparallel spin.

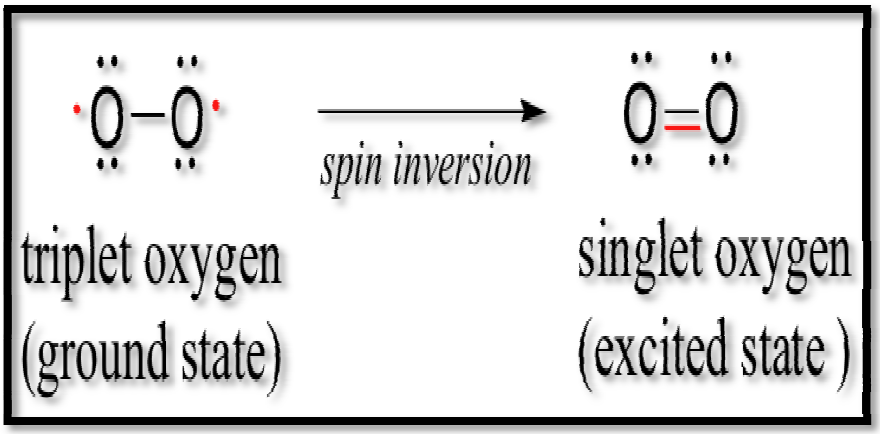


Fig. 2: Electron spin conversion[19]

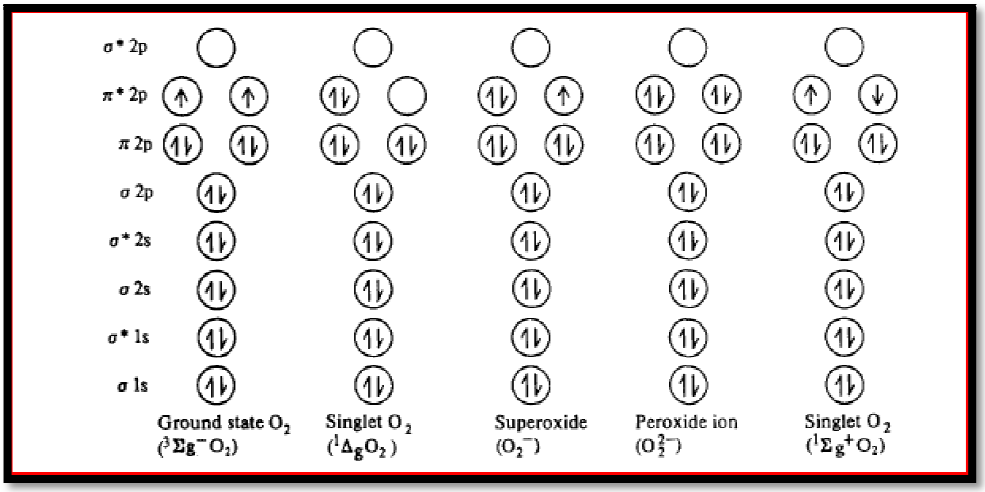


Fig. 3: Singlet states of O_2[19]

Molecular oxygen undergoes one-electron reduction at a time. When oxygen is reduced by a single electron the species formed is a superoxide anion, which is a FR:

$$O_2 + e^- \longrightarrow O_2^{\cdot -} \text{ (Superoxide anion)}$$

A "two-electron reduction" of oxygen would generate hydrogen peroxide, a ROS. The electron deficiency of the superoxide radical may be made up by the addition of a second electron to form peroxyl anion which interacts with two hydrogen ions resulting in hydrogen peroxide formation:

$$O_2 + e^- \longrightarrow O_2^{\cdot -}$$

$$O_2^{\cdot -} + 2H^+ \longrightarrow H_2O_2$$

Hydrogen peroxide may also be generated in biological systems through superoxide generating systems such as dismutation reaction in which the FR reactants give non-FR products.

$$2O_2 + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

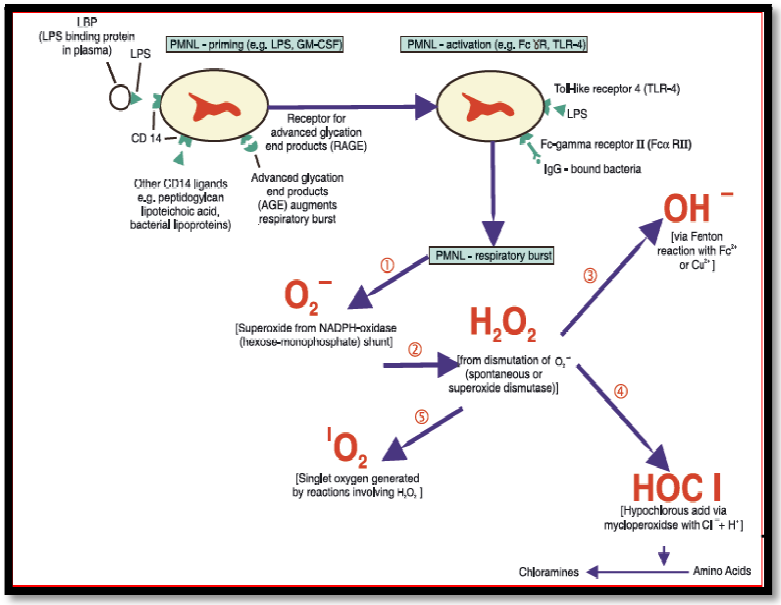


Fig. 4: Formation of hydrogen peroxide and its oxidative products [20,21]

Hydrogen peroxide plays a key role in free radical biochemistry. In the presence of transition metal ions, it can easily disintegrate to produce the hydroxyl radical (OH^-), one of the most reactive and damaging FR species.

The further reduction of hydrogen peroxide to water, involves the addition of two further electrons, producing the harmful hydroxyl radical (the "three-electron

reduction state"). This can occur in vitro by superoxide-driven **Fenton reaction**, or the metal (iron/copper) - catalyzed **Haber-Weiss reaction.** In these reactions, electrons are supplied by the oxidation of ferrous ions (Fe^{2+}) to ferric ions (Fe^{3+}) or cuprous ions (Cu^{+}) to cupric ions respectively (Cu^{2+}), respectively.[22]

The term **Fenton reaction** relates to the reaction between hydrogen peroxide and ferrous salts to produce a reactive species capable of oxidising a wide variety of organic substrates.[23]

The reaction has been studied in a large number of chemical and biochemical systems employing a variety of iron chelates and detection systems. The general finding is that most chelates cause oxidation of some but not necessarily all detectors.

$Fe^{+2} + H_2O_2 + Fe^{+3} + [H_2O_2]^{-} + OH + OH^{-}$ ⟹ Outer Sphere Mechanism

$L\text{-}Fe^{+2} + H_2O_2 + L\text{-}Fe(H_2O_2)+2$

$L\text{-}Fe(H_2O_2)^{+2} + L\text{-}Fe^{+3} + OH + OH^{-}$

$+ L\text{-}Fe^{+4} + 20H^{-}$ ⟹ Production of oxidizable substrate

The concept that the highly reactive hydroxyl radical (HO.) could be generated from an interaction between superoxide and hydrogen peroxide (H_2O_2) was proposed by Joseph Weiss in Professor Haber's final paper, published in 1934.

The **Haber-Weiss** reaction is a specific example of the Fenton reaction. It stated that the need for a metal ion catalyst is necessary for breaking down the hydroxyl radical into two chemical reactions.[24]

$$O_2^{-}.+ H_2O_2 \longrightarrow O_2 \quad +OH^{-} \quad OH^{\cdot}$$

ROS is a collective term which includes oxygen derived free radicals (ODFR) such as superoxide radical ($O_2^{\cdot-}$), hydroxyl radical ($^{\cdot}OH$), nitric oxide radical ($NO^{\cdot}$) species and non-radical derivatives of oxygen such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). ROS are synthesized by phagocytes during the respiratory burst mechanism. [25]

Cell metabolism involves the consumption of oxygen and its utilization via glycolysis to form pyruvate within the mitochondria and the amino acid cycle to produce ATP.

However, electrons leak from their transporters at a constant rate, reducing oxygen to the superoxide anion.

The incomplete reduction of oxygen is estimated at 1–2% of consumed oxygen and at a rate that exceeds the mitochondrial antioxidant scavenger's ability to eliminate superoxide.[26]

Functional production of superoxide radical involves the activation of the hexose-monophosphate (or NADPH-oxidase) shunt, that shunts glucose-6-phosphate from the glycolysis pathway and utilizes molecular oxygen and NADPH to form the superoxide radical anion (O_2^-).

Superoxide is only produced in the zone of the plasma membrane that is in contact with the phagocytosed particle.[20] Superoxide forms initially and then immediately dismutates to hydrogen peroxide by one of the three superoxide dismutase enzyme systems:[27]

- superoxide dismutase 1 – a Cu2+/Zn2+-dependent enzyme found within the cytosol;
- superoxide dismutase 2 – the Mn2+-dependent enzyme located within the mitochondria;
- superoxide dismutase 3 – extracellular enzyme, found at low levels extracellularly.

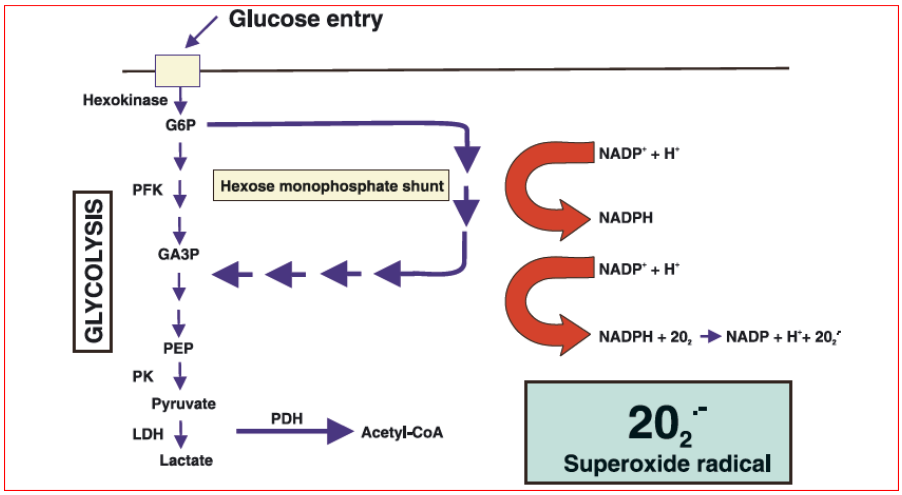


Fig. 5: A schematic representation of the NADPH-oxidase (hexose-monophosphate) shunt. [20]

The biology of ROS-mediated protein damage is highly vast and can lead to functional inactivation, which may be reversible or irreversible.

The cumulative effects of ROS on proteins are summarized as protein folding or unfolding (which may or may not be reversible);[20]

- protein fragmentation and polymerization reactions;
- protease degradation of the modified protein;
- formation of protein radicals;
- formation of protein-bound ROS;

- formation of stable end products e.g. carbonyl compounds such as oxo-acids or aldehydes (e.g. alanine to acetaldehyde)

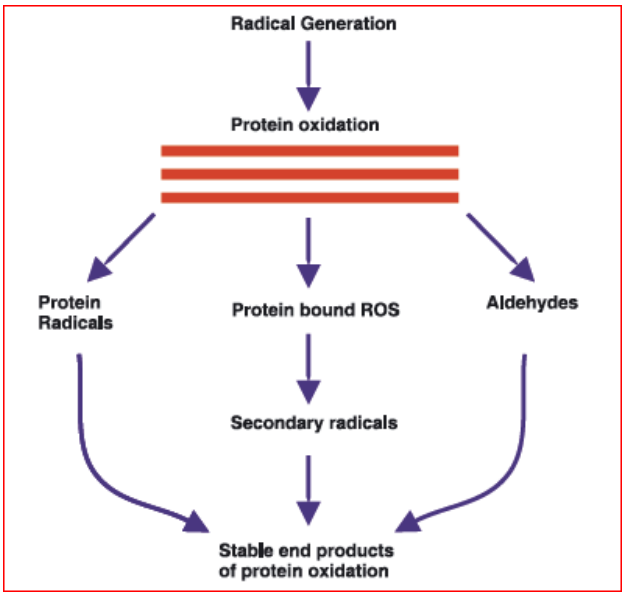


Fig. 6: Effect of free radicals and Reactive Oxygen Species on Nucleic Acids.[20]

Primary molecular sites for FR/ROS attack (mainly OH^-) are the heterocyclic purinic and pyrimidinic bases or the ribosyl and deoxyribosyl moieties. The effects could be attributed to the following effects[28]

- ✓ Sugar damage is initiated by hydrogen abstraction from one of the deoxyribose carbons and the predominant consequence is eventual strand breakage and base release.
- ✓ Another alteration at the sugar moiety is a β to α inversion at the 19-carbon, which disrupts the β-DNA structure.
- ✓ Radical attack on the bases results primarily in OH addition to the electron-rich double bonds, particularly the purine N-7–C-8 bond and the pyrimidine 5, 6 bond.

- ✓ Hydrogen abstraction from thymine-methyl groups.
- ✓ Attack at the DNA bases leading to as many as 50 base alterations.
- ✓ Another type of DNA damage mediated by iron *in vivo* is DNA protein cross-links, *e.g.* thymine-tyrosine

THE LINK BETWEEN OXIDATIVE STRESS AND PERIODONTITIS

IN ANIMAL STUDIES

Stahl et al., (1962)[29] conducted a study in the Department of Periodontia and Oral Medicine, College of Dentistry, New York, U.S.A. The aim of the study was to estimate the effect of a protein-free diet on the healing of gingival wounds in rats. In this study, 161 male Long-Evans strain rats weighing 354gm, were divided into five groups. Group I consisted of 36 rats that received a gingival injury by surgically stripping the gingiva mesial to the maxillary left first molar and were fed Purina Chow and water. Group II consisted of 36 rats that received a similar oral injury and were kept on a protein-free diet, consisting of cornstarch (70%), alphacil(15%), vegetable oil(10%), salt mixture No. 2 (U.S.P. 14)(14%), cod liver oil(1%) and Vitamin diet fortification supplement mixture of powdery consistency. Group III consisted of 10 rats that received a similar oral injury and were kept on a 27% casein diet of similar consistency as the protein-free diet. Group IV consisted of 25 rats that received the same oral injury which was allowed to heal for 28 days and were deprived of any edible supplements. Group V consisted of 54 rats that were placed on a protein free diet for 10 days and then re-fed with Purina Chow for the next 14 days. All these animals were sacrificed according to the following schedule : 30 min, 3, 6, 24, 48, 72, 96 hr, 5, 8, 12, 14, 18, 22, 26 and 30 days after injury except for those

animals belonging to Group IV who were sacrificed at the end of 28 th day following which they were decapitated and the maxillae was dissected out for the tissues to be fixed in formalin, to be prepared for histologic study. The results of the study state that those animals belonging to Group V depicted the most desirable healing event, as the experimental period progressed, inflammation persisted subepithelially causing bone resorption and in some specimens continuous wound breakdown was observed resulting in complete healing with little crestal bone growth. The study concluded that, the relative independence between alveolar bone repair and gingival soft tissue healing has been observed following nutritive therapy in animals.

Petelin et al., (2000) [30] conducted a study in the Department of Oral Medicine and Periodontology, University of Ljubljana, Slovenia. The aim of the study was to evaluate the influence of oxygen free radical scavengers on periodontal inflammation and healing process. In this study, experimental periodontitis was induced by the placement of elastic ligatures around the premolars (P2, P3, P4) and 1st molars (M1) in the upper and lower jaws of 15 beagle dogs, 9 months after the beginning of the experiment, the ligatures were removed. After 3 weeks of stabilization, all teeth were supragingivally scaled and the animals were divided into 3 groups of 5 dogs each. The 1st group received a liposome encapsulated superoxide dismutase (SOD), the 2nd group received a liposome-encapsulated catalase (CAT) and the 3rd group received both the enzymes encapsulated in liposomes. Four treatment modalities were tested in each group; i.e., supragingival scaling only (1), supragingival scaling and enzymes (2), supra- and sub-gingival scaling and root planing (3) and supra- and sub-gingival scaling and root planing with subgingival application of enzymes (4). The enzymes were delivered subgingivally on a daily basis for a period of 6 weeks. The clinical

parameters comprising of the Gingival index (GI), probing depth (PD), clinical attachment level (CAL), radiographic analysis and the histological evaluation were performed. The results indicated that the greatest suppression of gingival inflammation before ($GI = 1.8 \pm 0.1$) and after treatment ($\mathrm{GI} = 1.2 \pm 0.2$) ($\mathrm{p} < 0.003$) along with the reduction of PD ($\mathrm{PD} = 3.2 \pm 1.0$ mm before versus 2.0 ± 0.7 mm after treatment) ($p < 0.001$), and gain of CAL ($\mathrm{CA} = 3.0 \pm 1.7$ mm before versus $\mathrm{CAL} = 2.4 \pm 1.1$ mm after treatment)($p<0.001$) were observed. In addition to this radiographic analysis showed the greatest alveolar bone apposition in the group of teeth treated with scaling and root planing followed by the subgingival application of SOD or both enzymes ($p<0.001$) the results were statistically significant in all aspects. The authors concluded that the subgingival application of liposome-encapsulated SOD suppressed the periodontal inflammation, stimulated periodontal healing and alveolar bone apposition.

IN-VITRO STUDIES

Jacoby et al., (1991)[31] conducted a study in the Department of Periodontics, Baylor College of Dentistry, Dallas, Texas. The aim of the study was to ascertain the presence of "scavenging" enzyme superoxide dismutase (SOD) in the periodontal soft tissues adherent to the root surfaces of human teeth. In this study, the periodontal soft tissues were cleaved from sixteen freshly extracted human teeth, extracted for either orthodontic purposes or because of the presence of carious lesions. These teeth included 6 cuspids, 8 premolars, and 2 molars. The tissues were then prepared for subsequent biochemical and morphological studies and were divided into the following 3 groups with 16 samples belonging to each group. According to the study, the groups were segregated as follows, in Group 1) the samples were subjected to

immediate immersion in liquid nitrogen for the biochemical assay of SOD; Group 2) the samples were subjected to immediate fixation for routine transmission electron microscopy; Group 3) the samples were subjected to immediate fixation prior to preparation for electron microscopic immunohistochemistry. The results indicated that the human periodontal ligament (mean ± s.d = 4487 U ± 360 U) contained about twice as much SOD activity as human skin (mean ± s.d = 2249 U ± 70 U), but considerably less enzyme activity than that observed in the red blood cells (mean ± s.d = 25553 U ± 180 U). The study also concluded that periodontal SOD activity appeared to decrease with age and immunohistochemistry localized the SOD enzyme activity to the periphery of matrix collagen fibrils and to the glycocalyx of tissue fibroblasts.

Firatli et al., (1994)[32] conducted a study in the Department of Periodontology, University of Istanbul, Qapa, Istanbul, Turkey. The aim of the study was to investigate the antioxidant activities of commercially available antiseptics namely, chlorhexidine, listerine, sanguinarine and cetylpridinium chloride to that of oral antibiotics such as tetracycline HCl and doxycyline. In this study, aliquots for estimation were prepared from homogenate samples derived from the dura matter of Bovine brain and the solutions to be tested were added in different volumes (0.1 ml, 0.2 ml, 0.3ml, 0.4 ml) together with the corresponding volumes of phosphate saline. The prepared aliquots were subjected to the rinses over an hour for varying volumes namely, 0.1 ml, 0.2 ml, 0.3 ml and 0.4 ml and then subjected to sphectrophotometric assessment at 532nm. The results of the study stated that Doxycyline has the highest antioxidant activity in lower volumes (0.1 ml) followed by sanguinarine and Listerine. Tetracycline HCl, had similar effects in higher volumes (0.3 and 0.4 ml). The results

concluded that in addition to their antiseptic or antimicrobial effects, these preparations possess an antioxidative effect as well.

IN HUMAN STUDIES

Henry et al., (1984)[33]conducted a study in the Department of Periodontics, Mahidol University in Bangkok, Thailand. The aim of the study was to determine the phagocytic activities and the opsonisation intensities of PMN using chemi-luminescence (CL) on the cells derived from young adult residents in Bangkok. In this study, the study population consisted of young adults whose ages ranged from 18 to 35 years with a mean age range of (28.7 ± 4.58) yrs and were divided into the control and test groups based on the pre-existence of periodontal disease. The presence or absence of periodontal disease was determined by the Ramfjord index (1967) that revealed a three times greater attachment loss in the test subjects when compared to the control group (4.77 versus 1.56) respectively. Venous blood samples were collected from a control and test subject matched by sex and age. The neutrophils from all subjects were isolated and purified prior to the estimation by CL technique. This technique measures the activity of phagocytic cells that yields quantitative data in terms of light emission which correlates with the cell's oxidative metabolism. The light emission of CL can be enhanced by luminol and provides an indirect measurement of phagocytosis. The results of the study state that, PMN's produced significantly-higher CL peak values (peak CL= $315.5 \pm 33.1\%$) in the test group than that of the neutrophils of healthy-paired controls (peak $\mathrm{CL} = 403.7 \pm 44.0\%$) when each population of cells was challenged with zymosan pre-opsonized with autologous serum. The study concludes that, peripheral PMN from young periodontitis patients have oxidative metabolic activities similar to subjects free of

this disease, and that the enhanced PMN metabolism of patients may be due to a corresponding increase of serum opsonins.

Guarnieri et al., (1991)[34] conducted a study in the Department of Biochemistry and Periodontology, University of Bologna, Italy. The aim of the study was to determine the relationship between the 0_2^- generating activity of crevicular PMN and the antioxy-radical ability of GCF from healthy and chronic periodontitis (CP) patients. This study included 2 groups, Healthy group, that comprised of 16 healthy volunteers as subjects (control) with no clinical evidence of gingivitis (gingival index close to ***0''***) and no radiographic signs of bone loss (mean age range =25-27 yrs) and the CP group that comprised of 14 patients with radiographic evidence of generalized alveolar bone loss and probable pocket depths in excess of 4 mm at more than 4 sites; (mean age range = 49-62 yrs). GCF was collected by washing the gingival crevices and 20 µL was used for the PMN count. The release of 0_2^- by isolated blood PMN or by crevicular PMN was determined by the superoxide dismutase-inhibitable reduction of cytochrome C. The results of the study state that there was spontaneous generation of superoxide in the GCF of periodontitis subjects, with no differences in the antioxidant scavenging capacity between test group $= 103.8 \pm 9.0\%$ and control group $= 77.5 \pm 9.8\%$. The circulating PMN of control subjects did not show a spontaneous formation, differently from Chronic Periodontits (CP) patients. However, the results concluded that, no difference was reported in the SOD activity in GCF of healthy and periodontitis subjects.

Katsuragi et al., (1997)[35] conducted a study in the Department of Periodontics, Nippon Dental University, Niigata City, Japan. The aim of the study was to estimate the levels of metallothionein (MT) in patients with CP who are categorized into

smokers and non-smokers. In this study, the study population consisted of 33 male patients diagnosed with advanced periodontitis based on the clinical findings of the presence of periodontal pockets more than 7 mm in depth and a tooth mobility score of 3. In this study, the smokers (n = 22; age = 30 to 67 years) were defined as those individuals who habitually smoked at least 10 cigarettes per day at the time of the initial examination. The non-smoking group (n = 11; age = 41 to 70 years) consisted of individuals who did not smoke and had never smoked. The results of the study reported that smokers with periodontitis exhibited increased levels of MT value (value = 2.1 ± 0.9%), when compared to non-smokers with periodontitis (1.6 ± 0.8%). The authors conclude that the increased level of MT in smokers indicates a protective response to the increased inflammation in these patients.

Nishida et al., (2000) [36] conducted a study in the Department of Oral Biology, School of dental medicine, State university of New York, Buffalo, NY. The aim of the study was to determine the possible changes in GCF antioxidant defence status in chronic adult periodontal disease and also to investigate the nature of the local radical scavenging mechanisms with particular reference to glutathione and also to evaluate the association of Vitamin C on periodontal disease. The study comprised of a sample of **12,419** adults **(20-90+** years of age) from whom the periodontal parameters including the measurements of gingival bleeding, calculus, PD and CAL along with dietary requirements were recorded. The relationship between dietary Vitamin C and periodontal disease for the overall population was established by the multiple logistic regression analysis. The results obtained showed that there was a dose-response dependent relationship between the levels of dietary Vitamin C and periodontal disease with **OR of 1.28, 95% CI: and an OR of 1.30** for those taking **0-29 mg** of

Vitamin C per day. The results also stated that, the mean Vitamin C intake for males (119.76 ± 2.57 mg) was higher when compared to females (96.19 ± 1.79 mg) for the same age group (20-39yrs) and a modest but statistically significant association was observed between periodontal disease and low dietary Vitamin C intake ($p<.0001$), hence the authors concluded that the intake of low levels of Vitamin C exhibit the greatest risk of acquiring periodontal disease.

Sculley et al., (2002)[37] formulated a mini-review in the Centre for Healthcare Education, University College Northampton, Boughton Green Road, Northampton that emphasised upon stimulation of oxidative stress by bacterial antigens and cytokines like IL-8 that promotes the PMN to express adhesion molecules and move out of the circulation to the site of infection. It was also estimated that these PMN's produce proteolytic enzymes such as elastase, but also release O_2^- via the oxidative burst channel catalysed by NADPH oxidase. PMN's in periodontal patients display an increased number, adhesion and oxidative activity. The authors concluded that, superoxide is released into the phagosomal enclosure and into the extracellular surroundings. As the superoxide released is not target-specific, damage to host tissue also occurs.

Brock et al., (2004)[38] conducted a cross-sectional study in Periodontal Research Group, School of Dentistry, University of Birmingham, UK. The aim of the study was to determine both local (saliva and GCF) and peripheral (plasma and serum) antioxidant capacity in periodontal health and disease. The study comprised of twenty non-smoking volunteers with chronic periodontitis, sampled together with twenty age- and sex-matched, non-smoking controls. The clinical parameters assessed were PD and BOP% in both the study groups. After overnight fasting, saliva (whole

unstimulated and stimulated), GCF and blood were collected. The total antioxidant capacity (TAOC) was determined by using an enhanced chemiluminescence method. The results of the study stated that there were no significant differences in salivary flow rates (stimulated or unstimulated) between periodontitis subjects and controls ($p>0.1$) and GCF volumes were significantly greater in the disease group when compared to the healthy group ($p<0.001$). Mean PPDs of sampled sites was 3.91mm (range 1–9 mm) and whole-mouth pocket depths were 3.98 ± 1.08mm 3.98 (range 1–9 mm). Whole mouth percentage of sites that bled on probing in the periodontitis group was 22.6% (range 9%– 36.5%), and 41.67% (range 0%–100%) for the 6 sampled sites. GCF volume showed a significant positive correlation with PPD in the periodontitis group ($p<0.0001$). The study concluded that the antioxidant capacity of GCF is both qualitatively and quantitatively distinct from that of saliva, plasma and serum. Reduced plasma total antioxidant defence could result from low-grade systemic inflammation induced by the host response to periodontal bacteria, or may be an innate feature of periodontitis patients.

Panjamurthy et al., (2005)[39] conducted a study in the Department of Biochemistry and in the Department of Periodontology, Annamalai University, Annamalai Nagar, India. The aim of the study was to assess the degree of oxidative stress in patients with CP by measuring their levels of thiobarbituric acid reactive substances (TBARS), enzymatic antioxidants in the form of SOD, CAT and non-enzymatic antioxidants like Vitamin E and C and glutathione peroxide (GSHPx) in the peripheral venous blood samples. In this study, 25 patients diagnosed with chronic periodontitis receiving treatment were selected with an equal number of healthy subjects being enrolled for the same. The severity of periodontitis was diagnosed by

measuring the periodontal pocket depth (>3.5mm), gingival recession (Grade III), furcation development and degree of tooth mobility. Blood samples were obtained by venous arm puncture and collected in heparinized tubes. The levels of TBARS and non-enzymatic antioxidants, and the activities of enzymatic antioxidants in the patients plasma, erythrocytes and gingival tissues were assayed using specific colorimetric methods. The results of the study state that, the periodontitis sufferers had a significantly higher TBARS level (plasma = 10.34 ± 1.9 = n mol/ml, erythrocytes = 8.79 ± 2.1 pmol/mg 8.79 ± 2.1 Hb, erythrocyte membrane = 1.06 ± 0.13 = n mol/mg protein, gingival tissues = 170.6 ± 24.8 = 170.6 ± 24.8 nmol/100 mg protein) than the healthy subjects (plasma = 4.79 ± 0.834.79 ± 0.83 n mol/ml, erythrocytes = 3.65 ± 0.52p mol/mg Hb, erythrocyte membrane = 0.39 ± 0.06n mol/mg protein, gingival tissues= 130.8 ± 16.9 nmol/100 mg protein). In the plasma (SOD_1= 5.62 ± 0.34U/ml, SOD_2 = 3.46 ± 0.383.46 ± 0.38U/ml, CAT_1= 0.83 ± 0.07 0.83 ± 0.07U/ml, CAT_2 = 0.52 ± 0.08 U/ml) erythrocytes (SOD_1 = 5.1 ± 0.82 5.1 ± 0.82U/ml, SOD_2 = 2.43 ± 0.29 2.43 ± 0.29 U/ml, CAT_1= 4.8 ± 0.514.8 ± 0.51 U/ml, CAT_2 = 2.14 ± 0.18U/ml) and gingival tissues (SOD_1 = 18.6 ± 1.6 18.6 ± 1.6 U/ml , SOD_2 = 12.7 ± 1.4 12.7 ± 1.4 U/ml, CAT_1 = 11.9 ± 1.311.9 ± 1.3 U/ml, CAT_2= 7.6 ± 0.547.6 ± 0.54 U/ml) of the periodontitis sufferers, enzymatic antioxidant activities were found to be significantly higher, whereas the levels of non-enzymatic antioxidants were significantly lower (except for reduced glutathione in the gingival tissues) relative to the parameters found for healthy subjects with $p<0.001$. The authors conclude by stating that the disturbance in the endogenous antioxidant defence system due to over-production of lipid

peroxidation products at inflammatory sites and can be related to a higher level of oxidative stress in patients with periodontitis.

Alkalin et al., (2007)[40] conducted a study in the Department of Periodontology, Hacettepe University, Ankara, Turkey; to evaluate the Malondialdehyde (MDA) levels and total oxidant status (TOS) in serum, saliva and GCF in patients with CP. In this study, Thirty-six CP patients and 28 periodontally healthy controls were included. The periodontal status of all individuals was detected by measurements of PD, CAL, gingival index (GI) (Loe & Silness 1963), gingival bleeding index (GBI) (Muhlemann & Son 1971) and plaque index (PI) (Silness & Loe 1964). Full-mouth periapical radiographs were taken to determine the level of periodontal bone loss of the patients. All the samples (serum, saliva and GCF) were collected 48 h following the clinical measurements in the morning following an overnight fast. The participants were asked not to eat or drink anything. The results stated that the saliva (0.1 ± 0.007 µM) and GCF (0.88 ± 0.18 µM) MDA levels and serum (0.06 ± 0.16 µM H_2O_2 Equivalent) saliva (0.07 ± 0.21 µM H_2O_2 Equivalent) and GCF (3.52 ± 0.72 µM H_2O_2 Equivalent) GCF-TOS (39.1 ± 5.95 µM H_2O_2 Equivalent) values were significantly higher in the CP group than the control group, serum (0.58 ± 0.16 µM), saliva (0.04 ± 0.14 µM) and GCF(1.78 ± 0.42 µM) (1.78 GCF-TOS (31.40 ± 5.54 µM H_2O_2 Equivalent) ($p<0.05$), no significant difference in serum MDA levels was found ($p>0.05$). Correlations between clinical parameters and MDA and TOS levels in serum, saliva and GCF were investigated together for all individuals. Statistically significant, strong and positive correlations were observed between clinical parameters and MDA and TOS levels in saliva and GCF, statistically significant yet weak correlations were observed between clinical parameters and

serum TOS values ($p<0.05$). In addition, statistically significant strong positive correlations were observed between serum, saliva and GCF MDA and TOS levels ($p<0.05$). The results also revealed that MDA significantly increased locally in the periodontal pocket/oral environment, while TOS displayed both systemic and local increases in periodontitis. The study concluded by revealing that, increased MDA and TOS levels may play an important role in the pathology of periodontitis, and are closely related to the clinical periodontal status.

Akalin et al., (2009)[41] conducted a study in Department of Periodontology, Faculty of Dentistry, Karadeniz Technical University,Trabzon, Turkey. The aim of the study was to investigate the relationship between systemic and local antioxidant (AO) defense levels and periodontal status in pregnant women with CP. Serum and GCF TAOC, SOD concentrations and periodontal clinical parameters were measured in pregnant women at the beginning and the end of pregnancy and compared to values in women who were not pregnant women. In this study, One hundred twenty-four women, consisting of 33 pregnant patients with CP (PCP) in the first trimester (PCP1), 18 pregnant patients with gingivitis (PG) in the first trimester (PG1), 21 pregnant women with periodontal health (pregnant controls; P-controls) in the first trimester (P-control1), 27 non-pregnant patients with chronic periodontitis (CP), and 25 non-pregnant women as controls, were enrolled in this study. Periodontal examinations were performed and GCF/serum samples were obtained from 33 pregnant patients with CP (PCP), 18 pregnant patients with gingivitis (PG), and 21 periodontally healthy pregnant controls (P-controls), monitored in the first and third trimesters of 27 non-pregnant women with CP and 25 non-pregnant control women. The concentrations of TAOC (automated measurement method) and SOD

(spectrophotometric method) were determined. The periodontal parameters included the PD and CAL, gingival index (GI), gingival bleeding index (GBI) and plaque index (PI). The results stated that, all clinical parameters were significantly higher in PCP, PG and CP groups than in the control group ($P < 0.05$). PD (3.77 ± 0.35) and CAL (4.01 ± 0.36)(4.01 were significantly higher in the PCP group than in PG $(PD = 2.82 \pm 0.20)$ (PD= 2.82, $(CAL = 3.08 \pm 0.20)$ and P-control groups $(PD = 2.06 \pm 0.19)$, $(CAL = 2.18 \pm 0.24)$ $(P < 0.05)$. (PD= 2.06In the pregnant groups, all clinical parameters except PI increased significantly in the third trimester $(PI = 0.83 \pm 0.41)$ (PI = 0.83compared to the first trimester $(PI = 0.72 \pm 0.36)$ $(P < 0.05)$ and GBI was considerably higher in the PG3 group $(GBI = 2.46 \pm 0.49)$ (than the CP group $(GBI = 1.42 \pm 0.70)$ $(P < 0.05)$. (The average GCF-TAOC values were lower in the pregnant groups than in the periodontally matched, non-pregnant groups and were lower in the periodontitis groups than in the control groups $(TAOC = 0.41 \pm 0.08) < (TAOC = 0.40 \pm 0.11) < (TAOC = 0.59 \pm 0.10)$. In the pregnant groups, the levels decreased significantly from the first trimester to the third trimester. Statistically significant, strong negative correlations were observed between the clinical parameters and serum and GCF TAOC and SOD levels in pregnant women in both trimesters and in the non-pregnant groups. When the groups were evaluated together, the authors concluded by stating that, systemic and local GCF AO levels decreased in pregnancy and periodontitis and AO defence reached the lowest levels in the last phase of pregnancy, whereas periodontal status deteriorated. These results suggest that reduced AO capacity may be associated with adverse periodontitis–pregnancy interactions and each situation can be a provocative risk factor for the other.

Koshi et al., (2012)[42] conducted a study in the Department of Periodontics, Sree Mookambika Institute of Dental Sciences, Kulasekharam, K. District, Tamil Nadu, India. The aim of the study was to evaluate and compare the blood glucose, superoxide dismutase, glutathione peroxidase, nitric oxide synthase enzyme in Type-II diabetic patients with and without periodontitis. The study comprised a total number of 45 subjects consisting of both males and females (males – 28 and females –17) aged between 35 to 58 years recruited from the outpatient department of General Medicine of Sree Mookambika Institute of Medical Sciences. The study participants were divided into three groups of 15 participants each. Group I consisted of 15 healthy subjects, Group II consisted of 15 subjects with Type II diabetes mellitus with periodontitis and Group III of 15 subjects with Type II diabetes mellitus without periodontitis. The periodontal parameters recorded included, PI (Sillness and Loe), mSBI (Mombelli et al), PD and CAL, 5ml of venous blood sample was collected from all 45 subjects and it was then centrifuged in order to obtain serum. The estimation of glucose and antioxidant enzymes were performed using the standard biochemical methods. The results of the study stated that, there was a significant difference in the Plaque index (PI_1 = 0.23±0.00), (PI_2 = 0.54±0.020), (PI_3 = 2.56±0.24), mSBI ($mSBI_1$ = 6.47 ± 0.35%), ($mSBI_2$ = 8.54±0.28%), ($mSBI_3$ = 48.92±0.26%). Probing depth (PD_1= 1.15±0.01mm), (PD_2= 2.83±0.56mm) , (PD_3= 6.34±0.28mm) and Clinical level of attachment values (CAL_1= 1.15±0.01mm), (CAL_2= 2.45±0.16mm), (CAL_3= 6.18±0.04mm) between the three groups ($p<0.02$). There is a significant increase in superoxide dismutase (SOD_1= 3.67±1.23U/ml), $(SOD2 = 4.13 \pm 0.89\ U/ml), (SOD3 = 8.34 \pm 1.34\ U/ml)$, Nitric oxide synthase $(NOS1 = 5.89\ 0.46\mu M/L), (NOS2 = 9.85 \pm 0.45\ \mu M/L), (NOS = 317.34 \pm 0.87\ \mu M/L)$(NOS and decreased glutathione peroxidase

enzyme levels (GPx_1=22.56±0.24U/L), (GPx_2=18.16±0.15 U/L), (GPx_3=12.21±0.34 U/L) in Type-II diabetes patients with periodontitis ($p<0.03$). These results indicate a strong parametric relationship between oxidative stress biomarkers and periodontal disease. The study can be concluded on the note that, patients with chronic periodontitis show higher oxidative stress which is evident from the levels of biochemical oxidative stress markers.

Aziz et al., (2013)[43] conducted a study in Grant Medical College and Sir JJ Group of Hospitals, Mumbai, India, to estimate and compare some biochemical oxidative stress markers in patients with chronic periodontitis and healthy controls and to assess the effect of nonsurgical periodontal therapy on these parameters. The study included Eighty-two chronic periodontitis patients and 120 healthy controls, each divided into group I and group II. The periodontal status was evaluated using GI, PI, papillary bleeding index (PBI) and CAL. The biochemical parameters estimated were TAOC, antioxidant enzymes such as RBC-superoxide dismutase (RBC-SOD) and GPx, Vitamin C, malondialdehyde (MDA) and C-reactive protein (CRP) were also evaluated. Nonsurgical periodontal therapy (scaling and root planing; SRP) was performed on group II patients and a follow-up was done after 3 months. Results showed that group II patients showed higher inflammatory manifestations and oxidative stress, in terms of RBC-SOD levels (524.3 ± 81.9U/g Hb), MDA levels (4.1 ±0.4), CRP levels (3.4 ± 0.4) when compared to the group I controls, RBC-SOD levels (439.6 ± 75.9 U/g Hb), MDA levels (2.0± 0.3), CRP levels (1.8 ± 0.3) and the average baseline clinical parameters were significantly higher ($p < 0.001$) in group II patients (PI=2.2 ± 0.5), (GI=2.3 ± 0.5), (PBI=2.6 ± 0.4), (CAL= 7.7 ± 0.9) when compared to group I patients (PI=0.4 ± 0.2), (GI=0.7 ± 0.1), (PBI=0.9 ± 0.8), (CAL=

1.8 ± 0.3) following SRP therapy all clinical and biochemical parameters reduced significantly ($p < 0.001$) compared to their corresponding baseline values in group II patients ($p < 0.001$) (PI=1.3 ± 0.5), (GI=1.5 ± 0.5), (PBI=1.6 ± 0.4), (CAL= 6.2 ± 1.0).The authors concluded by stating that patients with chronic periodontitis showed higher clinical periodontal damage, systemic oxidative stress and inflammation when compared to healthy controls and also mentioned that the SRP therapy helps in lowering inflammatory burden and improving systemic oxidant: antioxidant imbalance.

Kanzaki et al., (2017)[44] conducted a review in the School of Dental Medicine, Tsurumi University, Yokohama, Japan and discussed the cytoprotective effects of SOD and its close association with CP. In this review article, it has been stated that the levels of SOD observed in the serum, saliva and GCF is elevated during periodontitis, however it can be brought back to normalcy by the augmentation of anti-oxidants in the form of nutrient supplement which has proven to produce a statistically significant reduction ($p<0.001$). Furthermore the authors conclude by stating that the genetic mutation of SOD is considered to be a risk factor for periodontitis. These data support a role for SOD as a potential diagnostic marker for periodontitis.

There are difficulties inherent in detecting and quantifying ROS directly in any disease, most research has concentrated on measuring the products (biomarkers) generated by ROS reacting with lipids, DNA or proteins within the body.[45]

There are a variety of generally accepted biomarkers, a number of which have been studied in the context of the periodontal diseases, amongst which SOD is most routinely assessed.[46,47,48]

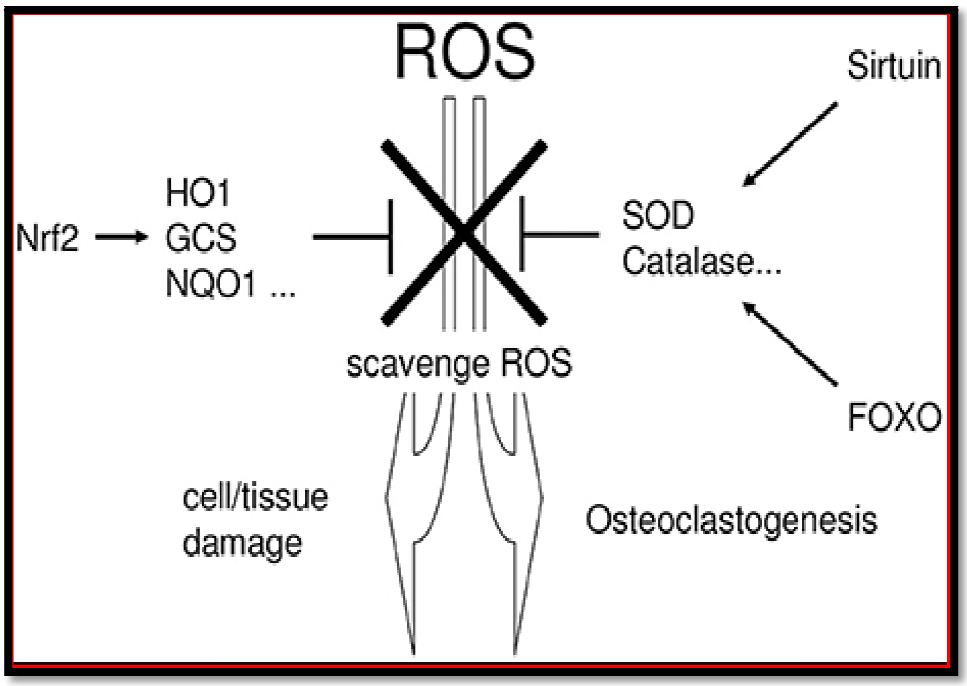


Fig. 7: The Regulatory Pathways of ROS[44]

THE LINK BETWEEN SUPEROXIDE DISMUTASE AND CHRONIC PERIODONTITIS

IN HUMAN STUDIES

Akalin et al., (2005)[49] conducted a study in the Department of Periodontology, Hacettepe University, Ankara, Turkey, to determine the SOD activity levels in the gingival tissue and GCF from patients with CP and periodontally healthy controls and also to analyse the correlation between gingival and GCF SOD activities. The study comprised of twenty-six CP patients and 18 healthy controls, the test group consisted of 14 women and 12 men (mean age 38.4 years; range 31–52 years) and the control

group included 13 women and five men (mean age 24.77 years; range 22–29 years). The periodontal status of the subjects were determined by measuring PD and CAL, and by recording GI, gingival bleeding index (GBI) and PI values. Full-mouth periapical radiographs were taken from the patients in order to determine the periodontal bone loss. In the study group, GCF was collected from teeth that presented with ≥5mm PD and 50% alveolar bone loss in right or left maxillary quadrant. The result of the study showed that all of the clinical parameters were significantly higher in the test group $(\mathrm{PD} = 3.80 \pm 0.09\mathrm{mm}), (\mathrm{CAL} = 4.60 \pm 0.15\mathrm{mm}), (\mathrm{GI} = 1.72 \pm 0.07), (\mathrm{GBI} = 0.94 \pm 0.04), (\mathrm{PI} = 1.85 \pm 0.11)$ 3.80 than the control group, $(\mathrm{PD} = 1.26 \pm 0.06\mathrm{mm}), (\mathrm{CAL} = 0.00 \pm 0.00\mathrm{mm}), (\mathrm{GI} = 0.00 \pm 0.00), (\mathrm{GBI} = 0.00), (\mathrm{PI}0.00 \pm 0.00)$ ($p < 0.05$). Mean gingival SOD activity levels in the CP group $(16.59 \pm 1.71$ (U/ml homogenate) was significantly greater than the control group $(3.73 \pm 0.22$ (U/ml homogenate). Correlations between gingival and GCF SOD activities were not statistically significant in either of the groups (p>0.05) nor were they significantly correlating it with the clinical parameters in either of the groups (p>0.05). The authors concluded by stating that SOD activity increases in the GCF in CP as a result of oxidative stress.

Baltaciog˘ lu et al., (2006)[50]conducted a study in the Department of Periodontology, Faculty of Dentistry, Karadeniz Technical University, Trabzon, Turkey. The aim of the study was to compare the serum and GCF-TAOC and SOD concentrations in post-menopausal patients with chronic periodontitis (PMCP) with those of pre-menopausal chronic periodontitis patients. In this study, a total of 114 women, consisting of 32 postmenopausal subjects with chronic periodontitis (PMCP) (mean age 48.15 ± 4.6 48.15 with an age range of 39–55yrs), 31 premenopausal

subjects with chronic periodontitis (mean age 37.4 ± 5.4 with an age range of 31 ± 47yrs) , 31post-menopausal periodontally healthy controls (PMPH) (mean age 49.1 ± 4.1 49.1 with an age range of 43 ± 55) 43and 26 pre-menopausal periodontally healthy controls (PH) (mean age 37.1 ± 4.2, 37.1with an age range of 30 ± 4430) were included in this study. The clinical parameters assessed were, PD, CAL, GI , GBI and PI. Full-mouth periapical radiographs were taken to determine the periodontal bone loss of the patients. The GCF samples were obtained from the patients at areas with deep pockets, between 08:00AM and 10:00AM. Venous blood was collected in plain tubes (for serum). Plain tubes were initially kept at room temperature for 30 min following which the respective samples were taken for complete analysis. After clinical measurements and samplings, serum and GCF TAOC and SOD concentrations were established in turn using an automated TAOC assay and spectrophotometric end point measurement. The results were analysed statistically. The results of the study stated that, all clinical parameters were significantly higher for the PMCP and CP groups than the control groups (post-menopausal and pre-menopausal; $p<0.05$), there was no significant difference between the PMCP and CP groups ($p>0.05$). The mean TAOC concentration in serum was 0.38 ± 0.14 0.38 in the PMCP patient group, 0.53 ± 0.190.53 in the CP patient group, 0.46 ± 0.14 0.46in the PMPH group and0.72 ± 0.21 in the PH group. The lowest values were observed in the PMCP group while the highest values were in the PH group. The mean serum SOD concentration was 0.92 ± 0.32 0.92 in the PMCP patient group, 1.18 ± 0.46 1.18 in the CP patient group, 0.96 ± 0.28 0.96 in the PMPH group and 1.67 ± 0.74 1.67in the PH group. Serum SOD concentrations in the PMCP, CP and PMPH groups were found to be significantly

lower than in the PH group ($p<0.05$). The mean GCF SOD activity levels were 0.43 ± 0.24 0.43 in the PMCP patient group, 0.82 ± 0.23 0.82 in the CP group, 1.11 ± 0.48 1.11 in the PMPH group and 1.26 ± 0.88 1.26 in the PH group. The PH group showed the highest values, while the lowest values were observed in the PMCP group. The study concluded that a decrease in systemic and local AO defence was observed owing to both menopause and periodontitis.

Canakci et al., (2007)[51] conducted a study at Department of Periodontology, School of Dentistry, Ataturk University, Erzurum, Turkey. The aim of the study was to investigate the total antioxidant capacity (TAC), SOD, glutathione peroxidase (GSH) activities and malondialdehyde (MDA) levels in serum, saliva, and GCF in preeclamptic and normotensive pregnant women with and without periodontal disease. The study comprised of 40 pregnant women who were divided into the following groups, Group I: consisting of 10 preeclamptic subjects with periodontal disease, Group II: consisting of 10 preeclamptic periodontally healthy subjects, Group III: consisting of 10 normotensive subjects with periodontal disease and Group IV: consisting of 10 normotensive periodontally healthy subjects. The clinical parameters included PD, CAL and bleeding on probing (BOP). All samples were obtained in the morning after an overnight fast and the subjects were asked not to drink (except water) or chew gum for the same period for accurate assessment. The results stated that there was significant differences observed in the clinical parameters between Group I and Group III patients with mean PD (4.24 ± 0.42), CAL (3.98 ± 0.42), PI $(69.7 \pm 21.7\%)$, BOP $(47.6 \pm 14.2\%)$ and PD (4.12 ± 0.38), CAL (3.87 ± 0.51), PI $(67.7 \pm 23.6\%)$, BOP $(45.6 \pm 13.4\%)$ respectively, ($p<0.001$). They also exhibited significantly higher percentages of sites with PD≥ 4 mm and sites with CAL

≥4 mm. The results also stated that there were significant differences observed in the biochemical values between Group II and Group IV ($p<0.05$) with the serum TAC $(1.15 \pm 0.29, 1.07 \pm 0.29)$, SOD$(3.26 \pm 0.69, 3.06 \pm 0.39)$, MDA$(3.29 \pm 0.89,$ $3.46 \pm 0.94)$, salivary SOD$(3.11 \pm 0.31, 3.21 \pm 0.21)$and GCF TAC $(0.16 \pm 0.04,$ $0.15 \pm 0.04)$, SOD$(1.44 \pm 0.37, 1.56 \pm 0.56)$, MDA$(2.99 \pm 0.32, 3.06 \pm 0.32)$ concentrations respectively. The authors concluded that systemic and local antioxidant and total antioxidant capacities are affected by periodontal disease in addition to the impact of preeclamptic status in women.

Agnihotri et al., (2009)[52] conducted a study in Department of Periodontics, Manipal College of Dental Sciences, Manipal, Karnataka, India. The aim of the study was to assess the influence of smoking on periodontal health, by estimating the levels of SOD enzyme in light and heavy smokers and also drawing comparison in the SOD levels between light and heavy smokers. The study population included 70 subjects in the age range of 20 to 55 years, of which 60 were smokers and 10 were non smoking controls. Clinical parameters like the PI, PD, and attachment loss (AL) were recorded. Smokers were divided into light smokers (<10 cigarettes/day) and heavy smokers (≥10 cigarettes/day) and into three subgroups: healthy, mild periodontitis, and moderate periodontitis. The GCF and saliva samples were collected from evaluated sites. SOD levels were analyzed using spectrophotometric assay. The results of the study state that, the mean levels of SOD in the GCF $(16.07 \pm 10.72\text{U/ml})$ and saliva $(20.21 \pm 14.93\ \text{U/ml})$ of smokers were decreased compared to controls $(\text{GCF} = 68.09 \pm 17.22\ \text{U/ml})$, $(\text{saliva} = 69.89 \pm 13.96\ \text{U/ml})$. Intra- and intergroup analyses showed a significant reduction in the levels of SOD in the GCF and saliva of heavy smokers $(\text{GCF} = 19.33 \pm 6.88\ \text{U/ml})$, $(\text{saliva} = 23.53 \pm$

$11.58\,\mathrm{U/ml}$) compared to light smokers ($\mathrm{GCF} = 34.43 \pm 9.38\,\mathrm{U/ml}$), ($\mathrm{saliva} = 35.51 \pm 15.93\,\mathrm{U/ml}$) and the control group ($\mathrm{GCF} = 68.09 \pm 17.22\,\mathrm{U/ml}$), ($\mathrm{saliva} = 69.89 \pm 13.96\,\mathrm{U/ml}$) ($p < 0.05$) . In this study the clinical parameters were not correlated with the biochemical values. The study concluded that there was a progressive reduction in SOD levels from healthy non-smokers to light smokers to heavy smokers and additionally proved that the benefits of reduced smoking and improved antioxidant levels may motivate smoking cessation.

Wei et al., (2010)[53] conducted a study in the Department of Stomatology, Nanjing Traditional Chinese Medical University, Changzhou Hospital, China. The aim of the study was to examine SOD, TOS and MDA levels in periodontal patients and to investigate the longitudinal effect of periodontal therapy on these indices in CP patients with varying degrees of periodontal destruction and inflammation. The study population included 83 individuals amongst which 48 were CP patients (27 males and 21 females) with an average age of 40.1 ± 7.3 years and the control group included 19 males and 16 females with an average age of (42.1 ± 7.7) years. The clinical parameters assessed were PD, CAL, GI, GBI and PI prior to and after 16 weeks post therapy. Full mouth periapical radiographs were taken to determine the level of periodontal bone loss. Whole salivary, GCF and serum samples were used in this study for the estimation of SOD, TOS and MDA. The results of the study state that, all the clinical parameters were found to be significantly higher in the CP group (PI= 1(0–3), (GI=1.76 (0.61–2.49), (GBI=2.71 (0.97–3.81), (PD=3.81 ± 0.44mm), (CAL=4.65 ± 0.91mm) compared with the control group (PI=0 (0–0), (GI=0 (0–0.3), (GBI= 2.71 (0.97–3.81), (PD=1.21 ± 0.23mm), (CAL=0.49 ± 0.33mm) ($p < 0.05$) before periodontal therapy and the levels of TOS (serum= 21.45 Mm H_2O_2

Equivalent), (saliva=9.12 Mm H_2O_2 Equivalent), (GCF=50.91 Mm H_2O_2 Equivalent) and SOD (serum=134.3 ± 22.19 U/ml), (saliva=216.4 ± 36.78 U/mg) ,(GCF= 561.2 ± 67.01 U/mg) values were significantly higher in the CP group than in the control group (serum= 15.44 Mm H_2O_2 Equivalent), (saliva=6.75 ± 1.02 Mm H_2O_2 Equivalent), (GCF=42.76 ± 7.94Mm H_2O_2 Equivalent) and SOD (serum=100.4 ± 19.23 U/ml), (saliva=174.9 ± 21.07 U/mg), (GCF= 476.3 ± 79.73 U/mg) ($p < 0.05$), the concentration of MDA in GCF was the highest among three samples (MDA_{GCF}= 1.03 ± 0.22 Mm) ($p < 0.05$) in CP patients before therapy and that in saliva was the lowest (MDA_{SALIVA}= 0.11 ± 0.05) ($p < 0.05$) regardless of group and therapy. Post-periodontal therapy, serum, saliva and GCF TOS (serum= 13.11 Mm H_2O_2 Equivalent), (saliva= 5.61 ± 0.95 Mm H_2O_2 Equivalent), (GCF=39.66 ± 3.08 Mm H_2O_2 Equivalent) and SOD (serum= 98.6 ± 19.08 U/ml), (saliva= 177.6 ± 24.61 U/mg), (GCF= 462.9 ± 76.3 U/mg) levels significantly decreased compared to baseline levels ($p < 0.05$). The results however were not significantly correlated with the clinical parameters in this study. The study concluded by stating that, lipid peroxidation status was higher in the periodontal region, with TOS and SOD increasing both locally and peripherally and that nonsurgical therapy can restore and control the subject antioxidant capacity by locally and systemically modifying the levels of MDA, TOS and SOD.

Kim et al., (2010)[54] conducted a study in the Department of Periodontology, Chonnam National University Hospital, Gwangju, Korea. The aim of the study was to compare the total antioxidant status (TAS) and SOD activity in the saliva of periodontally compromised patients before and after scaling and root planing (SRP) to assess their diagnostic utility. The study population comprised of a total of 26 patients

amongst which 14 patients were enrolled in the CP group and the remaining 12 in the periodontally healthy group. The clinical parameters assessed were PI, Sulcus bleeding index (SBI), PD, CAL and stimulated saliva samples were collected for assessment of the antioxidant status. Saliva sampling and clinical examination were performed at one week, one month and 3 months post SRP. The TAS and SOD activity in each patient's saliva was measured for comparative analysis between the groups. The results stated that in the test group, the mean probing depth (PD) and the clinical attachment level (CAL) significantly decreased from baseline ($\mathrm{PD} = 3.4 \pm 0.5\mathrm{mm}$), ($\mathrm{CAL} = 4.1 \pm 0.4\mathrm{mm}$) to one month post non surgical periodontal therapy over time ($\mathrm{PD} = 2.4 \pm 0.4\mathrm{mm}$), ($\mathrm{CAL} = 3.2 \pm 0.5\mathrm{mm}$) respectively. The average sum of the PI in the test group was 36.9, 8.1, 2.7 at the baseline, 1 week and 1 month after treatment, respectively, showing a significant decrease in accordance with the mean SBI being 44.1, 26.9, and 6.4 at baseline, 1 week and 1 month after treatment, respectively. The mean TAS in the test group was 335.7 ± 33.6 μM, 307.5 ± 50.2μM, 324.4 ± 46.0 μM and 326.8 ± 53.2 μM at the baseline, 1 week, 1 month and 3 months after treatment, respectively stating that the TAS activity decreased immediately after scaling and remained relatively constant with time . On the contrary the mean SOD activity of the test group was 41.4 ± 15.9%,36.4 ± 14.2%, 31.8 ± 22.9% and 53.2 ± 22.8% at the baseline, 1 week, 1 month and 3 months after treatment, respectively stating that SOD activity in the severe chronic periodontitis patients decreased immediately after SRP until 1 month but increased again at 3 months. The study concluded that, there was a significant difference in the total salivary antioxidant level between the periodontitis and healthy group during the experiment period. The total antioxidant level in the saliva was higher in the patients with severe chronic

periodontitis than the healthy prior to SRP. The SOD activity of the periodontitis patients was lower than the control at each time point.

Karim et al., (2012)[55] conducted a study in the Department of Periodontics, Annoor Dental College, Muvattupuzha, Ernakulam District, Kerala, India. The aim of the study was to analyse the activity of SOD enzyme and thiol antioxidants in the GCF and saliva as indicators of response to periodontal therapy. In this study, subjects were screened and randomly divided into three groups: 23 periodontally healthy controls, 24 with gingivitis, and 23 with periodontitis. Based on the clinical attachment levels, the periodontitis group was further divided into subgroups, including mild, moderate and severe periodontitis. GCF and saliva samples were collected for the estimation of SOD and thiol antioxidant concentrations at baseline and 15 days after nonsurgical treatment. The results of the study stated that, there was significant improvement in the clinical parameters especially observed in the pre and post therapy levels of PI (2.317 ± 0.444, 1.478 ± 0.416) and GI (2.317 ± 0.444 1.900 ± 0.367) and SOD was present in greater quantities in the GCF compartment (100.32±3.67 U/0.5 mL) than in saliva (39.99±3.52 U/0.5 mL), with elevated levels in mild and moderate subgroups as compared with severe periodontitis ($p<0.001$). Thiol concentrations were comparable in these media, 14.43±1.57 micromol /L in GCF and 15.09±2.26 micromol/L in saliva. Following treatment, SOD and thiol antioxidant concentrations significantly improved in all the patient groups and was well correlated with the clinical parameters with ($p<0.005$). The authors concluded that, the reduction of the inflammatory response following therapy resulted in improved antioxidant profiles in both the GCF and salivary compartments.

Sukhtankar et al., (2013)[56]conducted a study in the Department of Periodontics and Oral Implantology, Dr. DY Patil Dental College and Hospital, Pimpri, Pune, Maharashtra, India. The aim of the study was to estimate the effects of non-surgical periodontal therapy on SOD levels in gingival tissues of patients with CP. The study comprised of a total of 40 subjects out of which 20 were periodontally healthy and the remaining 20 were diagnosed with CP. Gingival tissue samples from CP patients were collected by excising the inner lining of the periodontal pocket at baseline and 2 months post therapy. In controls, tissue samples were obtained following tooth extraction scheduled for orthodontic reasons. Clinical parameters included PD, CAL, GI, BI and PI. SOD activities were assessed spectrophotometrically at baseline and 2 months post SRP. The results of the study stated that, there was improvement in the clinical parameters from baseline to 2 months post SRP in CP patients (PI=2.27 ± 0.31to 0.68 ± 0.37), (PD=6.7 ± 0.97to 5.7 ± 0.97), (CAL=6.65 ± 0.87to 5.7 ± 0.97), (GI=2.43 ± 0.32 to 0.94 ± 0.33), (GBI=17.81 ± 2.98 to 4.30 ± 1.89) and there was a drastic improvement in the gingival SOD levels from baseline to 2 months following SRP $(184 \pm 0.87\%)$ $(p < 0.001)$. The study concluded by stating that, non-surgical periodontal therapy significantly improves the clinical parameters and restores previously increased SOD levels to normalcy in CP patients.

Akpinar et al., (2013)[57] conducted a study in the Department of Periodontology, Cumhuriyet University Faculty of Dentistry, Sivas, Turkey. The aim of the study was to determine the effect of non-surgical periodontal therapy on GCF and serum oxidant–antioxidant levels in smoking and non-smoking patients with chronic periodontitis. The study comprised of 29 patients with chronic periodontitis

(15 smokers (CP-S) and 14 non smokers (CP-NS)) and 20 periodontally healthy subjects (10 smokers (H-S) and 10 non-smokers (H-NS)) totalling to 49 subjects being included in this study. GCF was collected from at least two pre-selected sites (one moderate and one deep pocket) in patients with CP. In the healthy group, GCF samples were collected from one, uniform site. Probing pocket depth (3-5mm to 2-4mm), CAL (7-10mm to 6-9mm), gingival (GI) (1-3 to 0-1) and plaque indices (PI) (1-3 to 0-1) and bleeding on probing (BOP%) (100%-6-9%) at the end of 6 weeks were measured. To determine serum total oxidant status (TOS) and total antioxidant status (TAS), venous blood was drawn from each subject. The GCF, serum sampling and clinical measurements were recorded at baseline and 6 weeks after periodontal treatment. The results of the study stated that there was statistically significant improvement in clinical parameters after periodontal treatment in both smokers and non-smokers group from baseline to 6 weeks ($p<0.005$). In the CP-S group, there were no significant differences in GCF TAS levels at both moderate and deep pocket sites between baseline and 6 weeks ($p > 0.05$). GCF-TAS levels in the CP-NS groups were significantly increased ($p<0.05$) at moderate (0.6 ± 1.6) and deep pocket sites (0.0 ± 0.1) between baseline (0.1 ± 0.0) (0.1and 6weeks (0.1 ± 0.2). (0.1GCFTOS levels in the CP-S groups were significantly decreased ($p < 0.05$) at deep pocket sites between baseline and 6 weeks. There was no significant difference in serum TAS levels of the all periodontitis patient groups between at baseline and 6 weeks ($p > 0.05$). Serum TOS levels in the CP-S and CP-NS groups were significantly decreased ($p < 0.05$) after periodontal treatments $(\mathrm{TOS-CP-S} = 0.5 \pm 5.7)$, $(\mathrm{TOS-CP-NS} = 1.2 \pm 7.8)$. (TOSThe study concluded that the periodontal treatment improves

the clinical parameters in both smokers and non-smokers. These results confirm that non-surgical periodontal therapy can reduce oxidative stress.

Muniz et al., (2015)[58] reviewed in Federal University Rio Grande, Brazil, the impact of antioxidant agents complimentary to periodontal therapy on oxidative stress and periodontal outcomes. The authors link CP and oxidative stress (OS) by stating that, CP is a multifactorial infecto-inflammatory disease caused by the interaction of microbial agents present in the biofilm associated with host susceptibility and environmental factors. OS is a condition that arises when there is an imbalance between the levels of FR and its antioxidant defences. This systematic review acknowledges the complimentary use of antioxidant agents to periodontal therapy in terms of oxidative stress/antioxidants, majority of the included studies were performed in chronic periodontitis patients. The study also states that, Lycopene, Vitamin C, Vitamin E capsules with fruits/vegetables/berry and dietary interventions were the antioxidant approaches employed. Only the studies that used lycopene and Vitamin E demonstrated statistically significant improvement when compared to a control group in terms of periodontal parameters.

VITAMIN -E AS A POTENT ANTIOXIDANT

INTRODUCTION

Vitamin E is a fat soluble Vitamin that was initially called tocopherol (greek: Tocos= childbirth, piro= to bear and ol= alcohol). Vitamin E was discovered in 1922 by Herbert McLean Evans and Katharine Scott Bishop and was first isolated in the pure form by Gladys Anderson Emerson in 1935 at the University

of California, Berkeley. Erhard Fernholz elucidated its structure in 1938 and shortly afterwards the same year, Paul Karrer and his team first synthesized it.[59]

The first use for Vitamin E as a therapeutic agent was conducted in 1938 by Widenbauer, who used wheat germ oil supplement on 17 premature newborn infants suffering from growth failure. Eleven of the original 17 patients recovered and were able to resume normal growth rates.[60]

It refers to group of compounds that include both tocopherols and tocotrienols. The molecules that contribute to the biological activity are four tocopherols and four tocotrienols, identified by the prefixes alpha- (α-), beta- (β-), gamma- (γ-), and delta- (δ-) however the nutritional content of Vitamin E is determined by its α-tocopherol content. It proves to be lipid-soluble antioxidant functioning within the glutathione peroxidase pathway that protects the cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction. The oxidized α - tocopheroxyl radicals produced in this process may be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol or ubiquinol.[61]

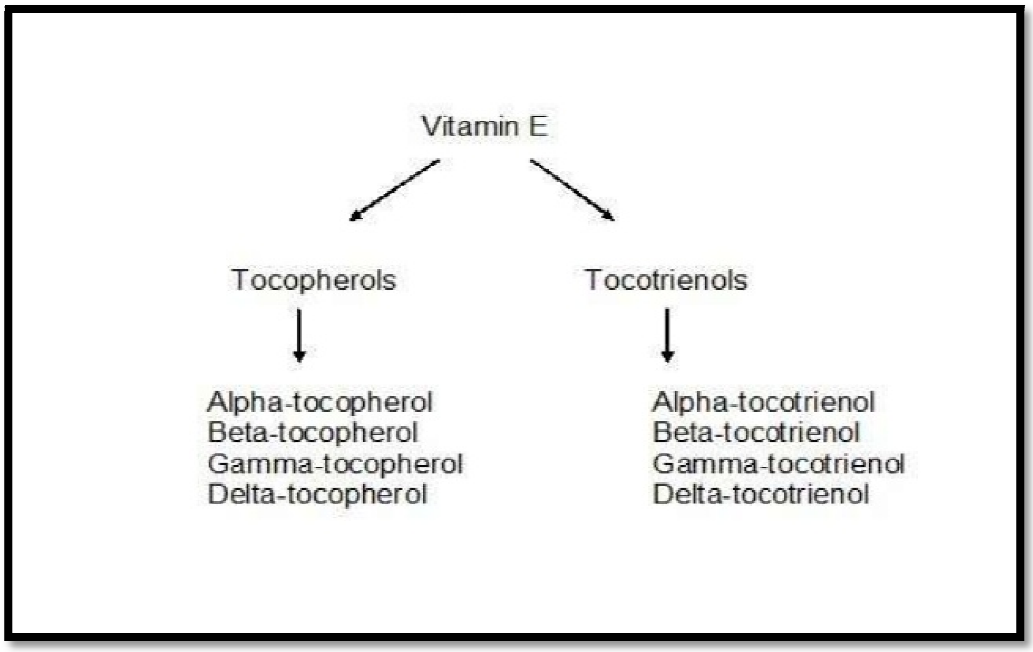


Fig. 8: Classification of Vitamin E[59]

α-tocopherol, the most biologically active form of Vitamin E, is the second-most common form of Vitamin E in the diet. This variant can be found most abundantly in wheat germ oil, sunflower oils.

As a fat-soluble antioxidant, it interrupts the propagation of reactive oxygen species that spread through biological membranes or through a fat when its lipid content undergoes oxidation by reacting with more-reactive lipid radicals to form more stable products.

Recently, studies have pointed to oxidative stress as being part of the pathogenesis of periodontal diseases. In order to combat this pathogenic process various externally supplemented antioxidants are being used. Non-enzymatic antioxidants are secondary mechanisms to neutralise ROS. Generally, these types of antioxidants are obtained exogenously, mainly through a balanced diet, which

included a variety of fruits and vegetables, such as blueberries, strawberries, grapes, avoca-do, tomatoes, spinach, and carrots.[58]

The non-enzymatic antioxidants are represented by fat-soluble Vitamin s (Vitamin A, Vitamin E-tocoferol and b-carotene), water-soluble Vitamin s (Vitamin C and Vitamin B complex), trace elements (zinc and magnesium), and bioflavonoids (plant derived) that have been supplemented for the suppression of oxidative stress produced in response to periodontitis.[58]

THE EFFECT OF VITAMIN E ON THE PERIODONTIUM

In Animal Studies

Kim et al., (1983)[62] conducted a study in the Department of Oral Medicine and Oral Pathology, Harvard School of Dental Medicine, Boston, to determine the effect of Vitamin E on the gingival wound healing in the albino rat. In this study, 60 young adult male and female albino rats (Sprague-Dawley Strain) were used as experimental animals and divided into 4 groups. Group I (20 animals) subjected to gingivectomy, Group II (20 animals) subjected to gingivectomy and supplemented with Vitamin E, Group III (10 animals) supplemented with Vitamin E and Group IV (10 animals) where no intervention was performed. The gingivectomy procedure was performed using a scalpel on the mandibular anterior marginal and attached gingiva. Of the 40 animals, 20 of them received 60 IU of d-alpha-tocopherol acetate daily, by mouth using a pipette. The remaining 20 did not receive the Vitamin E supplement following which gingival healing was studied grossly and histologically. The results of the study showed that complete healing of the gingiva was observed in both groups by 14 days. The animals belonging to the Vitamin E group depicted advanced healing

than the control groups, these animals showed pink gingiva covering most of the wound surface, whereas the control group still showed some redness and deformity at the gingival margin. The study concluded that, Vitamin E appears to accelerate wound healing during the early stages of granulation and epithelialisation.

Cohen et al., (1993)[63] conducted a study in the Naval Dental Research Institute, Building, Great Lakes, U.S.A. to evaluate the ability of Vitamin E supplementation to prevent periodontal destruction in rice rats, subjected to rotational stress. In this study, 32 male rice rats (57-84 days of age). One-half of the animals received a synthetic control diet, which included a standard 50 IU of Vitamin E/Kg feed. The remaining received a synthetic test diet that contained 5000 IU of Vitamin E/Kg feed. After 35 days of feeding on the synthetic diets, rats were assigned to normal or high-stress environments. 90 days after initiation of stress manipulations, animals were anesthetized and orbital blood samples were drawn for Vitamin E assessment. The primary dependent variable of interest was the CEJ-bone crest distance averaged across 14 sites (7 sites on each side). The results of the study showed that, the animals given high dietary Vitamin E had more than 4 times circulating Vitamin E than those given in low amounts. The study concluded that, neither Vitamin E nor stress condition had statistically significant effects but there was substantial bone loss observed in both the groups.

Asman et al., (1994)[64] conducted a study in the Department of Clinical Chemistry, Karolinska Institute, Huddinge University Hospital, Sweden, to elucidate the possible effect of antioxidants, such as Vitamin E and selenium, on collagen degradation. In this in vivo study, a mixture of Vitamin E and selenium (alpha-tocopherol acetate) was diluted in phosphate-buffered saline to permit injection

volumes of 0.1 to 0,5 ml. Sponges (15x15x4 mm), containing about 10 mg homologous H-collagen powder were implanted subcutaneously into the nape of the neck of Sprague-Dawley male rats weighing 250 g each. The rate of collagen degradation was monitored as the total radioactivity excreted between 8 and 18 days after implantation. Vitamin E and selenium were injected either subcutaneously in the back or directly into the sponges on these days. The in-growth of the granulation tissue into the sponges was studied by light microscopy of 2 sponges that had been implanted in each of 6 rats and divided in 2 rats per group. The results of the study stated that, by administering pharmacological doses of both Vitamin E and selenium subcutaneously and by injecting into sponges implanted subcutaneously, the breakdown of collagen was reduced. Injections in the sponges also arrested the maturation of the granulation tissue. Effects of subcutaneous administration of Vitamin E and selenium on 'H-colIagen degradation in experimental granulation tissue and total activity indicated that, the group of rats being subjected to 6.0 mg vit. $E + 120\mu g$ Se caused $13490 + 269$ E+120levels of collagen production in 2 rats that was statistically significantly ($p<0.005$). The results of the study concluded that, Vitamin E and selenium are potential inhibitors of the free oxygen radicals from phagocytic inflammatory cells and it also suggests that these radicals may play a role in the collagen destruction by granulation tissues as observed in periodontitis.

In Human Studies

Slade et al., (1976)[65] conducted a study in the University of Pennsylvania, School of Dental Medicine, Philadelphia, to determine the presence or absence of correlation between the serum levels of Vitamin E and periodontal disease. In this study, the study population included 24 subjects, 12 (5 males, 7 females) with

inflammatory periodontal disease and 12 (7 males, 5 females) without inflammatory periodontal disease. The groups were divided with respect to gingival periodontal index differences. The periodontal status of each patient was evaluated on the basis of clinical examination. Serum Vitamin E was determined from patients belonging to each group .Serum Vitamin E was measured by the microassay method and screening examination for periodontal disease was performed on the basis of O'Leary full mouth plaque estimation. All patients > 4.0 had clinical periodontal disease and those < 4.0 were without periodontal disease. The results of the study showed that, the gingival periodontal index had a significant difference ($p<0.01$) between both the groups, indicating the presence and absence of periodontal disease between the groups. The level of Vitamin E ranged from 0.71-1.38 mg/100ml for the group with periodontal disease and from 0.84-1.80 mg/100ml for the group without periodontal disease. The study concluded that there was no significant difference in the levels of serum Vitamin E between these 2 groups.

Prasad et al., (1980)[66] conducted a study in the Department of Biochemistry, University College of Science, Osmania University, Hyderabad, India to elucidate the effects of Vitamin E supplementation to human volunteers. This study was carried out in 13 adult male subjects ages between 25 and 30 years and five young boys ages between 13 and 18 years. Each subject received 300 mg of d/-a-tocopheryl acetate as tablets in three divided doses daily for a period of 3 weeks following which the plasma Vitamin E levels were estimated in these subjects by the thin layer chromatographic method. The results of the study stated that, the plasma Vitamin E levels varied between 4.6 and 1 1.7 μg/ml initially with a mean value of 7.7 μg/ml and increased to values in the range of 1 to 18.2 μg/ml at the end of 3 weeks of

supplementation. The increase in the level of Vitamin E was statistically significant he bactericidal activity of peripheral leukocytes showed a significant decrease from an initial value of 3.8 to 0.8 log reduction in viable counts after supplementation. The percentage of acid phosphatase activity released under particle stimulation was 9.2 ± 1.05 (mean ± SE) initially in seven subjects studied and showed a marginal but significant decrease to 6.9 ±1.51 after supplementation. The study concluded that, the effect of mega doses of Vitamin E comprising of 300mg, given daily for a period of 3 weeks produced a significant depression in the bactericidal activity of the leukocyte and the mitogen induced lymphocyte transformation.

Miller et al., (2005)[67] performed a meta-analysis gathering data within the geographical region of Seattle, Washington, to estimate the dose–response relationship between Vitamin E supplementation and total mortality by using data from randomized, controlled trials. The study population included 1,35,967 participants in 19 clinical trials. Of these trials, 9 tested Vitamin E alone and 10 tested Vitamin E combined with other Vitamin s or minerals. The dosages of Vitamin E ranged from16.5 to 2000 IU/d (median, 400 IU/d). The results of the studies stated that, 9 of 11 trials testing high-dosage Vitamin E (>400 IU/d) showed increased risk (risk difference > 0) for all cause mortality in comparisons of Vitamin E versus control. The pooled all-cause mortality risk difference in high-dosage Vitamin E trials was 39 per 10 000 persons (95% CI, 3 to 74 per 10 000 persons; P = 0.035). For low-dosage Vitamin E trials, the risk difference was -16 per 10 000 persons (CI, _41 to 10 per 10 000 persons; p > 0.2). A dose–response analysis showed a statistically significant relationship between Vitamin E dosage and all-cause mortality, with increased risk of dosages greater than 150 IU/d.

Singh et al., (2013)[68]conducted a study in Department of Periodontics and Oral Implantology, Post Graduate Institute of Dental Sciences, Rohtak to investigate the levels of SOD activity in serum and saliva of patients with CP and to assess the outcome of SRP with and without Vitamin E supplementation in terms of changes in periodontal parameters and SOD activity in patients with chronic periodontitis. The study population included a total of 60 individuals out of which 22 periodontally and systemically healthy individuals were recruited in the control group (CG) and 38 patients with CP were included in the test group. The test group was further divided based on the treatment being offered. Patients in treatment group-1 (TG-1) received SRP and in treatment group-2 (TG-2) received SRP along with oral administration of 200mg (300 IU) of Vitamin E (Evion) in the form of capsule every alternate day along with the meal for three months. The periodontal parameters included the PI, GI, BOP, PD and CAL. PI and GI were recorded on four sites while rests were recorded at six sites on each tooth. All the samples, prior to and after periodontal therapy were collected after 48 hrs of the clinical measurements. All participants, after an overnight fast were told to abstain from eating, drinking anything except water and brushing prior to sample collection the next morning. The participants were enquired whether they had followed these instructions before sample collection. The results of the study showed that biochemical parameters in control and test group at baseline and the evaluation of this cross-sectional data revealed that the SOD activity in saliva ($p<.0.05$) and serum ($p<0.001$) was markedly lower in disease samples as compared to the periodontally and systemically healthy subjects which was estimated to be 10.95%(saliva) and 59.82%(serum) in the test group and 21.66%(saliva) and 69.36%(serum) in the control group. After 3 months follow-up SOD activity

improved in both the treatment groups however, the improvement in TG-2 was high compared to TG-1 along with better improvement in periodontal parameters in TG-2. TG-1 and TG-2 show improvement in all the periodontal parameters after SRP was significantly high in TG-2 as compared to TG-1 (P< 0.05, 0.001, 0.001, 0.05 and 0.05 for PI (1.8300-0.400) GI(1.9400-0.6700), BOP%(67.350-6.1800), PPD(3.7300-1.8500) and CAL(4.12-2.34), respectively. Serum SOD levels in TG-2(85.90%) raised even above the level of control group(69.36%). The study concluded by stating that, systemic and local SOD levels are lowered in CP and adjunctive Vitamin E supplementation improved periodontal healing as well as antioxidant defence.

MATERIALS & METHODS

STUDY DESIGN AND PATIENT SELECTION:

This study was conducted in the Department of Periodontics and Oral Implantology, Sri Ramakrishna Dental College and Hospital, Coimbatore. 46 patients of age limit 25-60 years were recruited for the study. The study population included 17 patients with Chronic Periodontitis (CP) supplemented with Vitamin E, 17 patients with Chronic Periodontitis without Vitamin E supplementation and 12 systemically healthy individuals with clinically healthy periodontium who served as controls. All the patients except healthy controls were treated with full mouth scaling and root planing followed by evaluation after 6 weeks.

STUDY ETHICS AND SAFETY:

This clinical study followed the principles in the Declaration of Helsinki. Study was approved by Institutional Review Board and Ethical Committee of Sri Ramakrishna Dental College and Hospital, Coimbatore. Informed consent was obtained from each patient before enrolling them in the study. The subjects involved in this study had to satisfy the following criteria.

INCLUSION CRITERIA:

Group I: Healthy individuals (Control group)

- Absent of bleeding on probing.
- Probing depth (PD) < 4mm.
- Absence of clinical attachment loss.
- Patients without any systemic disease.

Group II: Chronic Periodontitis without Vitamin E supplementation

(Test group I)

- Probing depth (PD) ≥ 5mm.
- Presence of clinical attachment loss.
- Radiographic evidence of alveolar bone loss.
- Patients without any systemic disease.

Group III: Chronic Periodontitis with Vitamin E supplementation

(Test group II)

- Probing depth (PD) ≥ 5mm
- Presence of clinical attachment loss.
- Radiographic evidence of alveolar bone loss.
- Patients without any systemic disease.

EXCLUSION CRITERIA:

- Patients with history of antibiotics, anti oxidants, Vitamin supplements and anti-inflammatory drug intake for past 3 months.
- Patients who had undergone any form of periodontal therapy in past 6 months.
- Patients with history of smoking
- Pregnant and or lactating women.
- Patients without any underlying systemic disease.

ARMAMENTARIUM

DIAGNOSTIC AND OPERATING INSTRUMENTS:

1. Mouth mirror
2. Williams Periodontal probe
3. Explorer
4. Tweezers
5. Cheek retractor
6. Suction tip
7. Local anaesthetic solution-Lignocaine 2% with adrenaline 1: 80,000
8. 3 ml disposable syringe
9. Gauze pieces and customized cotton rolls
10. Gracey curettes
11. Ultra sonic scaler (EMS SCALER)
12. Evion 200mg (150IU) capsules
13. Hard gelatine capsules (placebo)
14. Enzyme Linked Immuno Sorbent Assay kit (ELISA) for analysing SOD
15. Microcapillary pipettes (for collecting GCF) (1- 10µl)
16. Labelled sterile vials for storing GCF
17. Labelled polypropelene wide-mouthed containers for storing the capsules
18. Aluminium foil

CLINICAL EXAMINATION:

On examining the patient, the following clinical parameters were recorded:

Plaque Index (PI):[69] Full mouth plaque score was recorded by using Plaque Index given by Silness and Loe (1967).

Pocket Depth (PD):

Conventional Probing Depth (Hill EG 2006):[70]

The probing pocket depth was assessed on each tooth from the gingival margin to the base of the sulcus using Williams periodontal probe at 6 specific sites

1. Distofacial line angle to the midline of distal surface
2. Mid Facial surface
3. Mesiofacial line angle to the midline of mesial surface
4. Distolingual line angle to the midline of distal surface
5. Mid Lingual surface
6. Mesiolingual line angle to the midline of mesial surface

Clinical Attachment Level (Philstrom BL 1992):[71] The Clinical Attachment Level was assessed on each tooth from the Cemento enamel junction (CEJ) to the base of the sulcus in all the 6 sites using Williams periodontal probe as mentioned for probing depth.

Bleeding On Probing (Mombelli et al 1987):[72] Bleeding on probing was assessed in each tooth using Mombelli's Modified Sulculs Bleeding Index.

RADIOGRAPHIC ASSESSMENT (Kallestal C 1989):[73]The radiographic evidence of bone loss was determined using full mouth intra oral periapical radiographs to assess if the distance from CEJ to the alveolar crest was >2mm.

GCF COLLECTION FOR SOD ESTIMATION:

GCF samples were obtained using microcapillary pipettes[74] in the morning following an overnight fast during which patients were requested not to drink (except water) or eat. GCF samples were collected from sites with maximum attachment loss for patients in Group II and Group III ; and from uniform sites in healthy individuals. The site was isolated for GCF collection with cotton rolls. The sulcular areas were gently air dried. A colour coded, calibrated (1-10µl) volumetric microcapillary pipette (Sigma-Aldrich Co, St. Louis, USA) ™ was placed at the entrance of the gingival crevice. The GCF samples which were contaminated with blood or saliva and air bubbles were discarded and fresh samples were collected. Each microcapillary pipette containing GCF was wrapped in aluminium foil and was placed inside separate sterile polypropelene tubes and stored at -80°C in an ultra low temperature freezer (Hera freeze-86°C basic upright freezers, Thermo Fisher Scientific India Pvt.td, Mumbai, India), until further analysis of SOD by ELISA.

SCALING AND ROOT PLANING (SRP):

After collecting GCF, complete scaling and root planing procedures were carried out under local anesthesia (2% lignocaine plus 1:80,000 adrenaline) with ultrasonic scaler (EMS SCALER) and Gracey curettes (Hu-Friedy) on the same day in Group II and Group III patients. Oral hygiene instructions were given to the patients and they were instructed to use chlorhexidine mouthwash (0.2%) 10 ml twice daily

for 2 weeks. Subjects in Group II were prescribed with a placebo pill (*Hard gelatine capsule) for a month and Group III patients were prescribed with Vitamin E supplements (Evion capsules, 200mg bid) for a month and Group II patients were prescribed with a placebo pill (*Hard gelatine capsule) mimicking the former, for the same duration. These pills were dispensed in a transparent wide-mouthed plastic container and the patients were also handed over a calendar to mark the dates of consuming the pill, for a month's duration.

FOLLOW UP:

After 6 weeks, patients were re-examined to observe the changes in the clinical parameters. GCF samples were also collected to observe the changes in the biochemical parameters that were measured earlier. The patients were also assessed in terms of their compliance by re-checking the calendar with the number of pills remaining in the container.

ELISA ANALYSIS FOR GCF SAMPLES:

The biochemical evaluations were done in a laboratory in Coimbatore. On the day of ELISA analysis, the GCF samples which were stored at -80°C were thawed and brought to room temperature. The ELISA buffer was added to the non-specific binding antigen (NSB) and 80 wells. Wells of plates pre-coated with polyclonal antimouse IgG antibodies were used. The wells were designated as blank; NSB (non-specific binding); B0 (maximum binding); TA (total activity) or standard (S1–S7), and samples. The samples and the standards were added to the wells. 2 µl of the GCF sample was transferred to 49 µl of ELISA buffer which was provided with the DNA/RNA Oxidative Damage ELISA kit (Bioassay Technology Laboratory), ™ by

passing the plunger through the micro capillary pipettes. SOD acetylcholinesterase (AChE) tracer was added to the wells except the blank and TA. Monoclonal antibody was added to appropriate wells except the blank, TA and NSB wells. The plate was covered with an adhesive strip and incubated for 1hour at 37°C after mixing. Each well was aspirated and washed five times using an autowasher. After washing, SOD AChE tracer was added to the TA wells and Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid) which contains the substrate to AChE, was added to each well, and the wells were placed at room temperature. Finally, the optical densities were read at 450 nm by a microplate reader. Corrected B0 (B0 – NSB) was determined using the optical densities and calculated as %B/B0 for each standard and samples. The samples were compared with the standards. The concentrations of SOD were expressed as International units/Litre (U/L).

DEPARTMENT OF PERIODONTICS

ELUCIDATION OF THE ANTIOXIDANT STATUS IN RESPONSE TO NON SURGICAL PERIODONTAL THERAPY AND POST VITAMIN E SUPPLEMENTATION IN PATIENTS WITH CHRONIC PERIODONTITIS – A RANDOMISED CONTROLLED TRIAL

PROFORMA

FORM I - SCREENING PROFORMA

NAME: O.P NO:

AGE: SEX:

OCCUPATION:

POSTAL ADDRESS:

TELEPHONE NUMBER/E-MAIL ID:

INCLUSION CRITERIA:

Group 1: Healthy individuals (Control group)

- Absence of bleeding on probing.
- Probing depth (PD) < 4mm.
- Absence of clinical attachment loss.
- Patients without any underlying systemic disease.

Group 2: Chronic Periodontitis (Test group I & II)

- Probing depth (PD) ≥ 5mm
- Presence of clinical attachment loss.
- Radiographic evidence of alveolar bone loss.
- Patients without any underlying systemic disease.

EXCLUSION CRITERIA:

- Patients with history of antibiotics, anti oxidants, Vitamin supplements and anti-inflammatory drug intake for past 3 months.
- Patients who had undergone any form of periodontal therapy in past 6 months.
- Patients with history of smoking
- Pregnant and or lactating women.
- Patients without any underlying systemic disease.

FORM II- HISTORY PROFORMA

Chief complaint with duration:

	Absent	**Present**
1. Bleeding gums	☐	☐
2. Bad breath	☐	☐
3. Pain in gums	☐	☐
4. Swollen gums	☐	☐
5. Pus discharge from gums	☐	☐
6. Mobility	☐	☐
7. Hypersensitivity	☐	☐

8. Any other complaint's (Specify):

PERSONAL HISTORY:

1. Brushing habit:
2. Drug history:

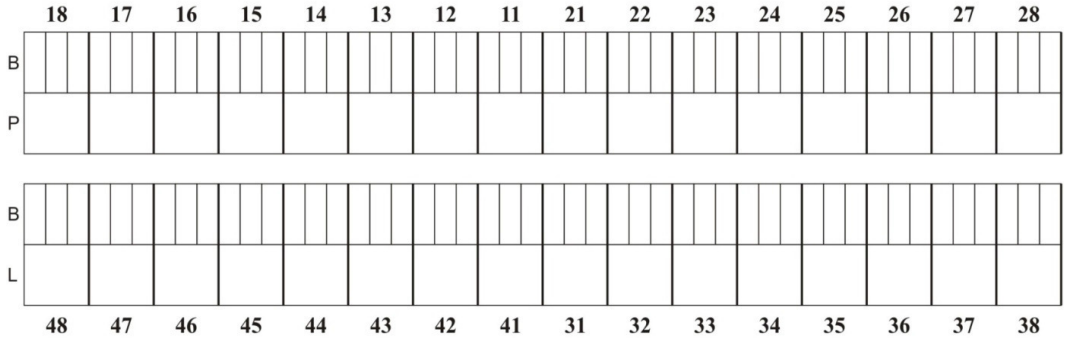
   Dosage:

   Duration:

FORM III- CLINICAL ASSESSMENT (Baseline)

DATE:_____

1. PLAQUE INDEX (SILNESS AND LOE 1967):



Calculation:

$$\frac{\text{Sum of score of each teeth}}{\text{Total number of teeth examined}}$$

Inference:

Excellent: 0

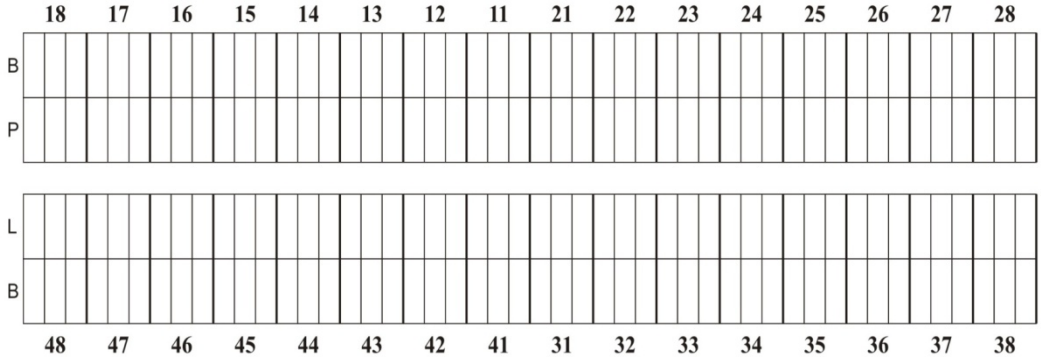
Good: 0.1 – 0.9

Fair: 1.0 – 1.9

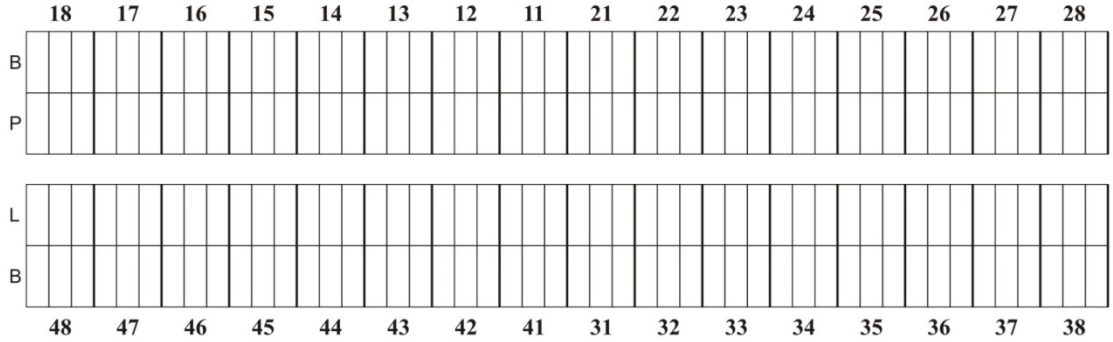
Poor: 2.0-3.0

2) PERIODONTAL STATUS

a) PROBING DEPTH (CONVENTIONAL PROBING METHOD)



b) CLINICAL ATTACHMENT LEVEL:



3) MODIFIED SULCULAR BLEEDING INDEX (Mombelli et. al, 1987)

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
48	**47**	**46**	**45**	**44**	**43**	**42**	**41**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**

$$\textbf{Bleeding on Probing \%} = \frac{\textbf{Total score obtained}}{\textbf{Maximum score obtained} \times \textbf{Total number of teeth examined}} \textbf{ x } 100\%$$

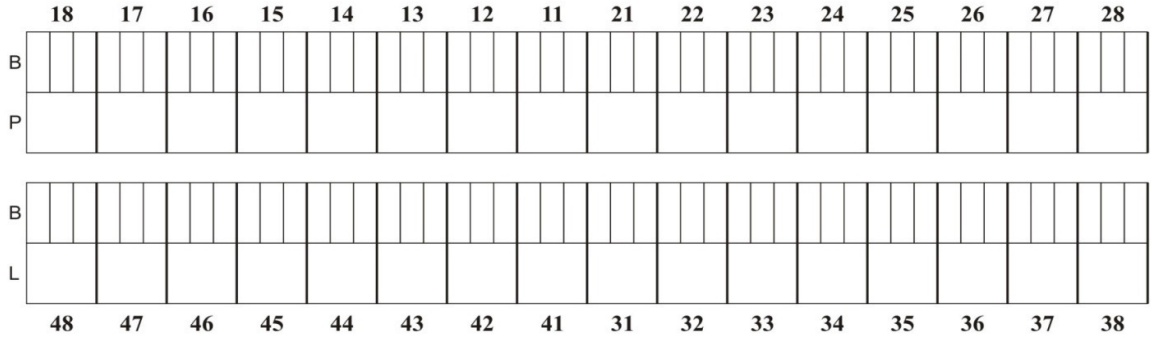
4) RADIOGRAPHIC ANALYSIS:

5) GCF Superoxide dismutase level –

FORM IV - CLINICAL ASSESSMENT (Post Operative)

DATE: _____

1) PLAQUE INDEX (SILNESS AND LOE 1967):



Calculation:

$$\frac{\text{Total Sum of score of each teeth}}{\text{Total number of teeth examined}}$$

Inference:

Excellent: 0

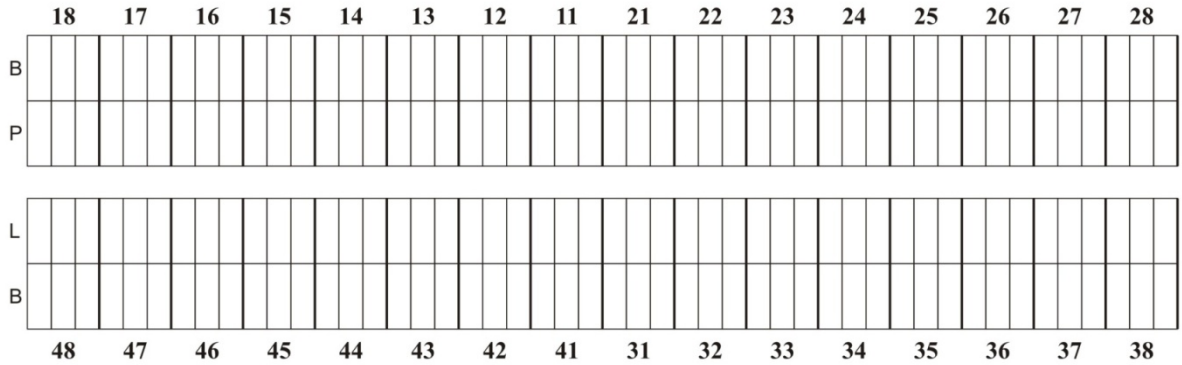
Good: 0.1 – 0.9

Fair: 1.0 – 1.9

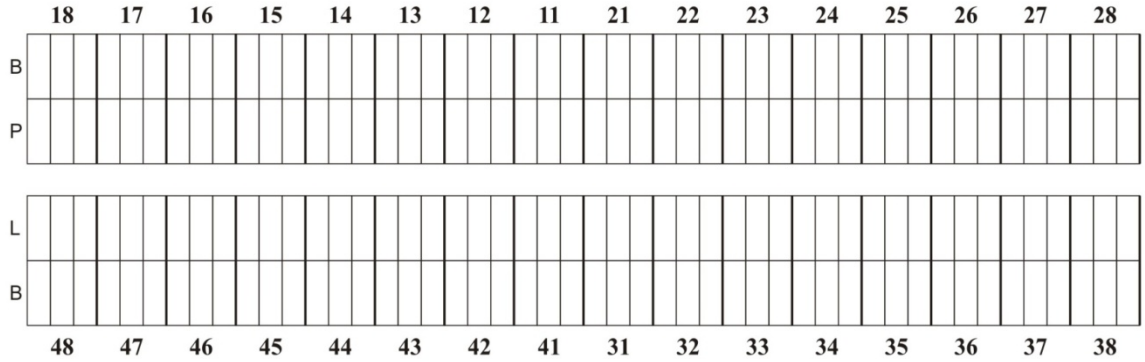
Poor: 2.0-3.0

2) PERIODONTAL STATUS

a) PROBING DEPTH (CONVENTIONAL PROBING METHOD)



b) CLINICAL ATTACHMENT LEVEL



3) MODIFIED SULCULAR BLEEDING INDEX (Mombelli et. al, 1987)

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

$$\text{Bleeding on Probing \%} = \frac{\text{Total score obtained}}{\text{Maximum score obtained} \times \text{Total number of teeth examined}} \text{ x } 100\%$$

4) RADIOGRAPHIC ANALYSIS:

5) GCF Superoxide dismutase level –

FORM V

CONSENT FORM

CERTIFICATE BY INVESTIGATOR

I certify that I have disclosed all details about the study in the terms easily understood by the patient.

Dated: _____ **Signature: _____**

Name: _____

CONSENT BY SUBJECT

I have been informed about the research protocol to my satisfaction and have been explained about the risks involved in this procedure. So, I'am willing to participate in the study and undergo non surgical periodontal therapy under local anaesthesia and collection of Gingival crevicular fluid (GCF)

Dated: _____ **Signature or thumb impression: _____**

Name: _____

ஒப்புதல் படிவம்

இந்த ஆராய்ச்சி நெறிமுறை மற்றும் இந்த செயல் அறையில் உள்ள ஆபத்துக்கள் குறித்து நான் தெரிவிக்கப்பட்டுள்ளேன். அதனால் இந்த ஆராய்ச்சியில் பங்குகொண்டு மயக்கமருந்து மூலம் அறுவைசிகிச்சை இல்லாத ஈறு சிகிச்சை மேற்கொள்ள ஈறு திரவம் சேகரிக்க சம்மதம் தெரிவிக்கிறேன்.

தேதி_____ கையொப்பம் அல்லது கை எண்ணம் _____

பெயர் :

FIGURES

Fig. 9: Armamentarium

Fig. 10: Healthy control (Baseline)

Fig. 11: GCF collection in healthy control (Baseline)

Fig. 12: Chronic Periodontitis at Baseline

Fig. 13: Collection of GCF in Chronic Periodontitis patient at Baseline using Microcapillary pipette

Fig. 14: Chronic Periodontitis at 6 weeks follow up

Fig. 15: Collection of GCF in Chronic Periodontitis patient at 6 weeks using Microcapillary pipette

Fig. 16: Vitamin E capsules

Fig. 17: Placebo capsules

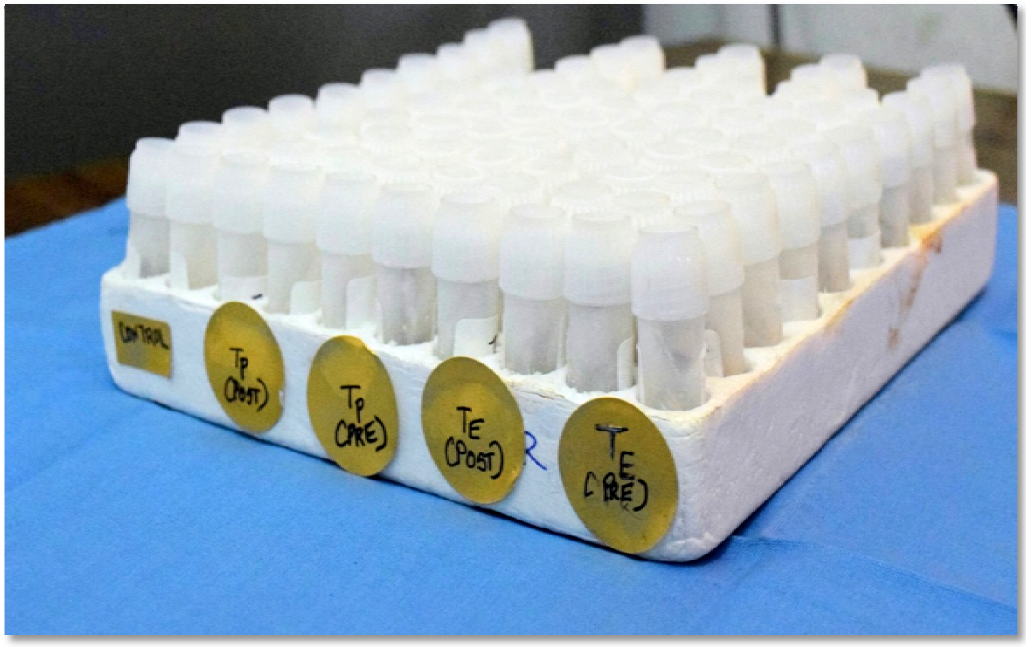


Fig. 18: GCF samples collected

Fig. 19: Transport medium

Fig. 20: Ultra low temperature freezer for storing collected samples

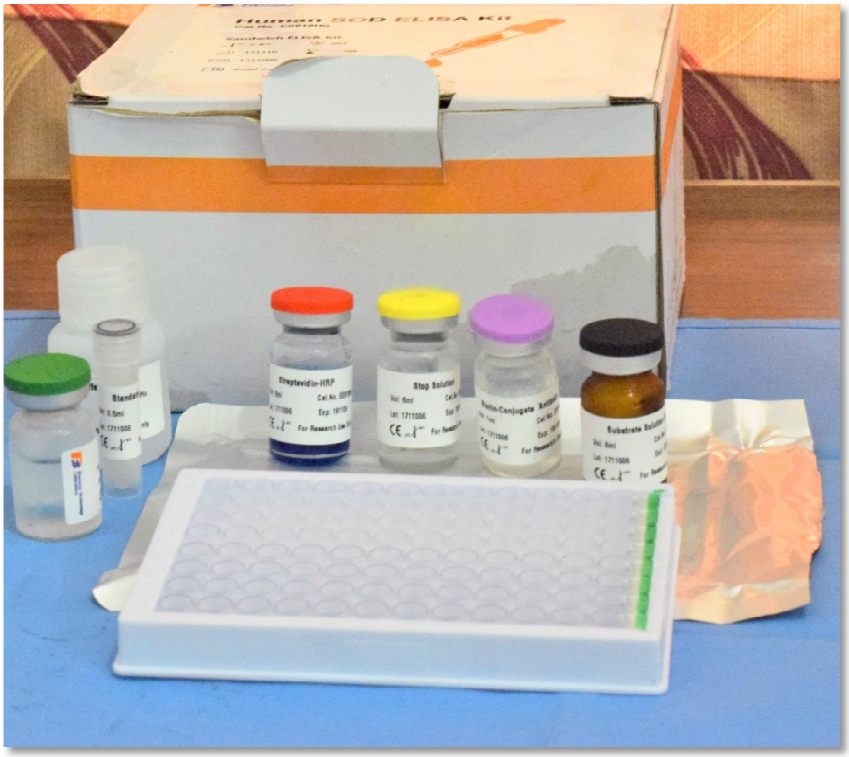


Fig. 21: ELISA kit (SOD)

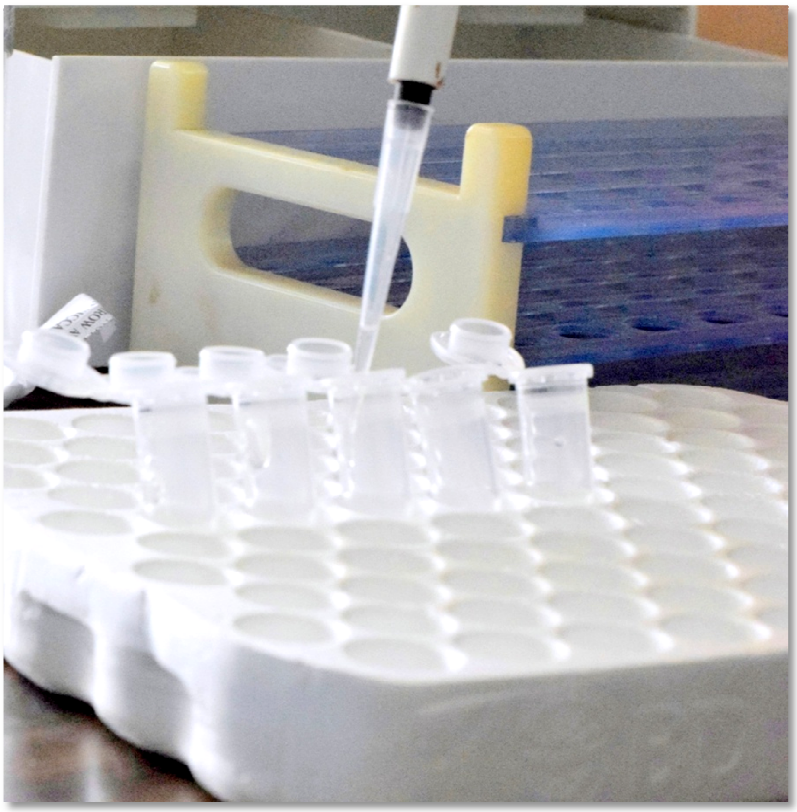


Fig. 22: Preparation of standard solution

Fig. 23: Addition of GCF sample to the strip well

Fig. 24: Addition of Streptavidin-HRP antibody to the strip wells

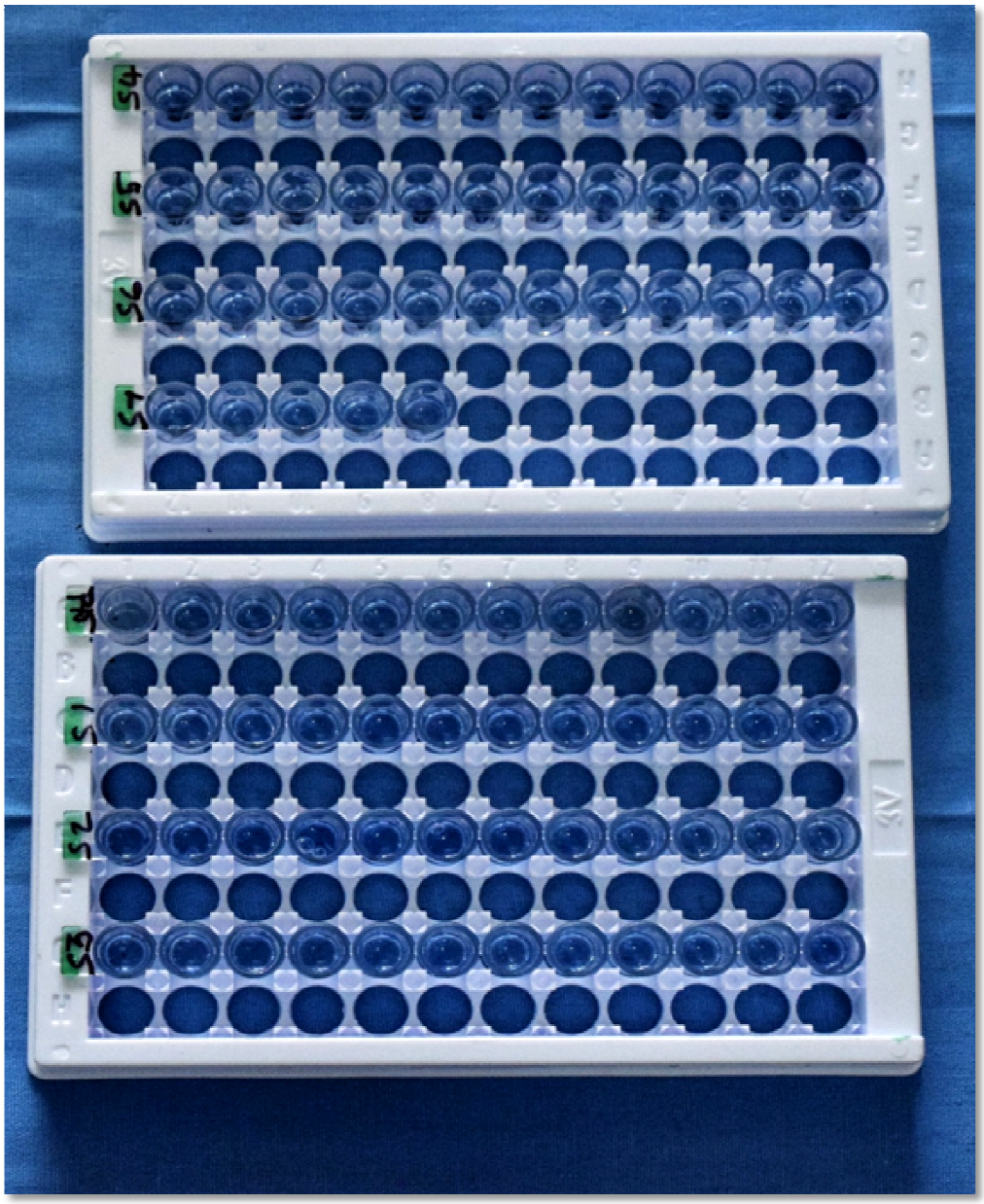


Fig. 25: Post addition of antibody to strip well

Fig. 26: Incubated for an hour

Fig. 27: Preparation of the wash buffer solution

Fig. 28: Serial washing of wells

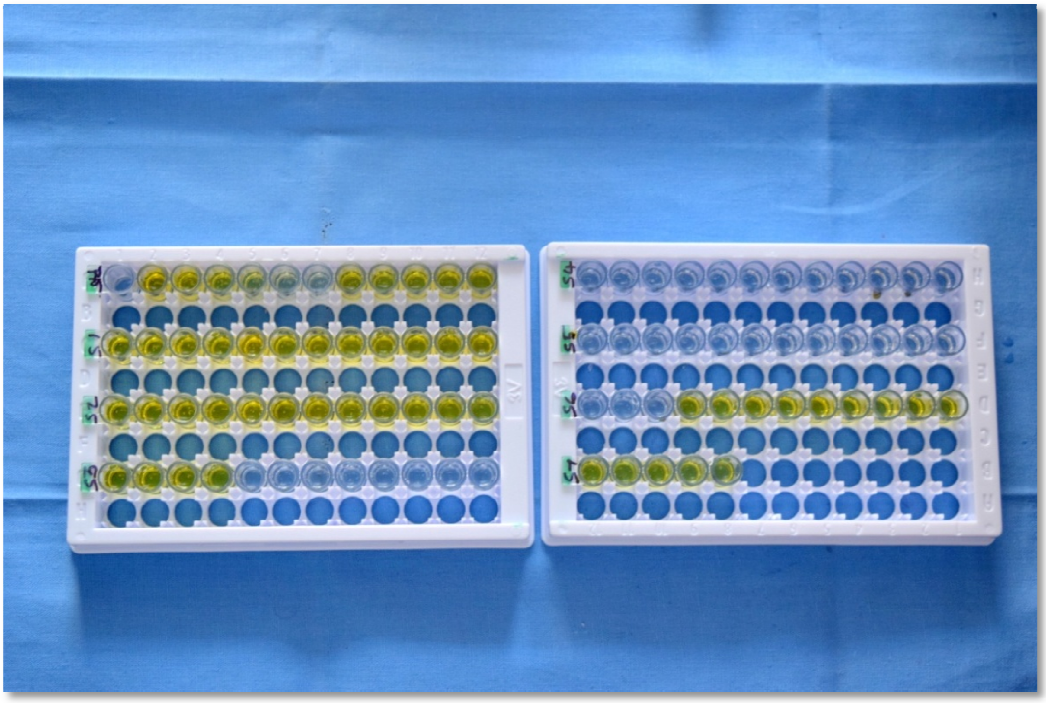


Fig. 29: Color change post addition of Ellman's Reagent

Fig. 30: ELISA reader for SOD estimation

Fig. 31: Reading the plate using ELISA reader

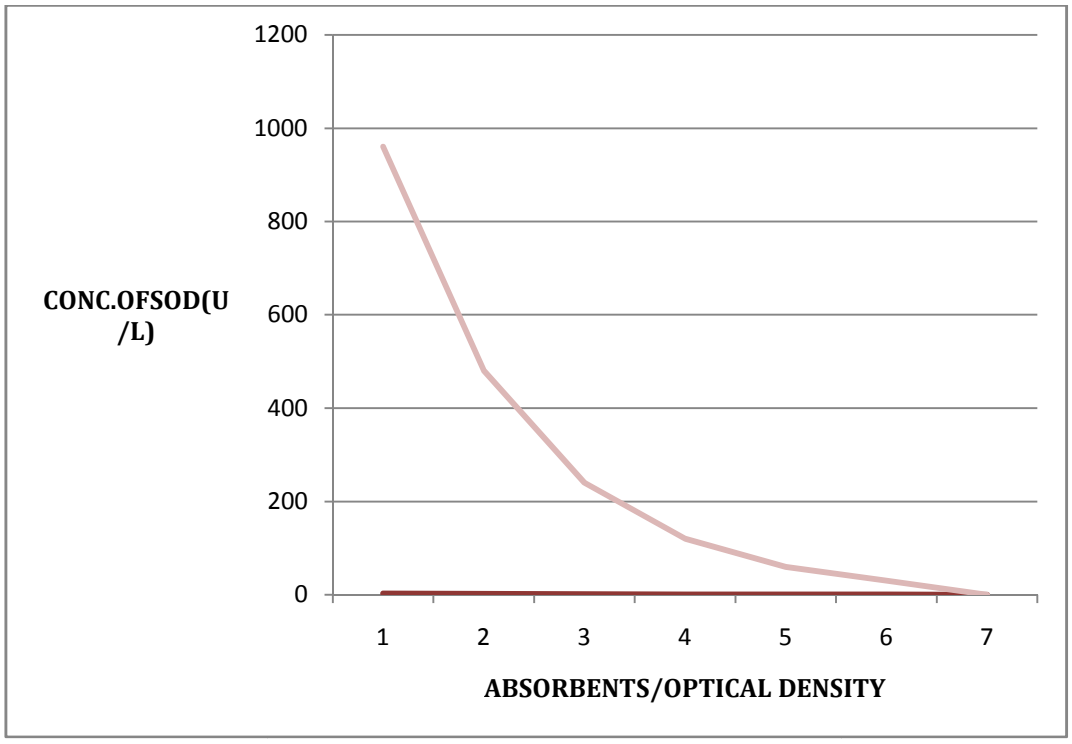


Fig. 32: Standard curve generated

RESULTS

Statistical analysis was performed using unpaired t-test, the independent difference in variables were assessed by paired t-test and the non-parametric Mann Whitney U Test. The baseline GCF levels of SOD and clinical parameters (PI, PD, CAL, and mSBI%) in Groups I, II and III were analysed and compared. At the recall visit, 6 weeks after SRP, the same comparison was performed for Groups II and III. The levels of SOD were recorded and compared for both these groups at baseline and at 6 weeks.

The various clinical parameters assessed in accordance with the demographic data and biochemical analysis are as follows:

a) DEMOGRAPHIC CHARACTERISTICS:

1) Age: Group I -The mean age of the healthy controls was 27.67±8.172 years (Table 1)

Group II - The mean age of the CP patients without Vitamin E supplementation was 44.47±9.951 years (Table1)

Group III - The mean age of the CP patients with Vitamin E supplementation was 44.53±9.07 years (Table1)

Group I -75%, 17% and 8% of the subjects belonged to the age groups of 21-30 yrs, 31-40 yrs and 41-50 yrs respectively.(Table 2),(Graph 1)

Group II - 6%, 29%, 47% and 18% of the subjects belonged to the age groups of 21-30 yrs, 31-40 yrs, 41-50 yrs and >50 yrs respectively.(Table 2),(Graph 1)

Group III - 41%, 41% and 18% of the subjects belonged to the age groups of 31-40 yrs, 41-50 yrs and >50 yrs respectively.(Table 2),(Graph 1)

2) Gender: Group I -The gender distribution noted amongst healthy controls was 17%- males and 83%-females (Table 3, Graph 2)

Group II - The gender distribution noted amongst CP patients not being supplemented with Vitamin E was 59%- males and 41%-females (Table 3, Graph 2)

Group III - The gender distribution noted amongst CP patients supplemented with Vitamin E was 53%- males and 47%-females (Table 3, Graph 2)

b) BASE LINE AND POST TREATMENT (SRP) CLINICAL PARAMETERS:

1) Plaque Index (PI):

The full mouth PI scores were recorded for all participants at baseline and after SRP in Groups II and III. At baseline, the mean plaque scores of Groups I, II and III were $0.367 \pm 0.208, 1.618 \pm 0.322$ and 1.512 ± 0.359 respectively (Table 4, Graph 3) Following SRP, the scores were reduced to 0.79 ± 0.446 and 0.663 ± 0.286 in Groups II and III respectively, which was statistically significant ($p<0.05$) (Table 4). The mean plaque score reduction between Group II (0.8282 ± 0.4882) and Group III (0.8488 ± 0.3858) was not statistically significant ($p>0.05$) (Table 5,Graph 8).

2) Probing Depth (PD):

The PD was measured by the conventional probing technique. Probing depths were recorded for all the participants at baseline and after SRP in Groups II and III. At baseline, the mean probing depths of Groups I, II and III were $1.628 \pm$

$0.609\text{mm}, 3.663 \pm 0.328\text{mm}, 3.762 \pm 0.422$ mm respectively (Table 4, Graph 4). Following SRP the scores were reduced to $2.786 \pm 0.337\text{mm}$ and $2.669 \pm 0.675\text{mm}$ in Groups II and III respectively which was statistically significant ($p<0.05$) (Table 4). The mean probing depth reduction between Group II (0.8771 ± 0.3364) and Group III (1.093 ± 0.469) was not statistically significant ($p>0.05$) (Table 5,Graph 9).

3) Clinical Attachment Level (CAL):

The CAL was recorded for all participants at baseline and post SRP in Groups II and III. At baseline, the mean clinical attachment levels of Groups I, II and III were 0.125±0.088mm, 2.682±0.825mm and 1.597±0.642mm respectively (Table 4, Graph 5). Following SRP, a significant gain in CAL was obtained in Group II ($2.014 \pm 1.071\text{mm}$) and Group III ($1.0165 \pm 0.552\text{mm}$) which was statistically significant ($p<0.05$) (Table 4). The mean gain in CAL observed in Group II (0.6682 ± 0.4429) and Group III (0.58 ± 0.4194) and was not statistically significant ($p>0.05$) (Table 5, Graph 10)

4) Modified Sulcus Bleeding Index (mSBI):

Bleeding on probing was observed in all patients at baseline and at the post treatment visit in Group II and Group III. The mean bleeding scores of Groups I, II and III were $7.911 \pm 5.866, 70.854 \pm 24.246$ and $63.784 \pm 13.873\%$ respectively at baseline (Table 4, Graph 6). Following SRP, bleeding on probing in Groups II and III were reduced to $39.303 \pm 22.113\%$ and $33.917 \pm 14.437\%$ respectively which was statistically significant ($p<0.05$) (Table 4).The improvement in the bleeding

scores observed in Group II (31.552±21.164) and Group III (29.867±14.199) was not statistically significant (p>0.05) (Table 5, Graph 11)

6) Estimation of SOD Levels:

Estimation of SOD was done in all patients at baseline and at the post treatment visit in Group II and Group III patients. At baseline, the mean SOD levels were 184.83 ± 135.71U/L, 602.51 ± 74.258U/L and 628.31 ± 83.268 U/L for Groups I, II and III respectively (Table 4, Graph 7). Following SRP, SOD levels in Groups II and III reduced to 507.01 ± 92.928 U/L and to 194.02 ± 96.594 U/L ($p < 0.05$) respectively, which was statistically significant. The reduction in SOD levels were observed to be 95.5 ± 74.028 U/L in Group II and 434.28 ± 100.309U/L in Group III which was statistically significant (p<0.05) (Table 5, Graph 12).

Table 1: Mean age group in Group I, Group II and Group III

	Mean	SD	Std. Error	95% CI for Mean Lower	Upper	Minimum	Maximum	Sig
GROUP 1	27.67	8.172	2.359	22.47	32.86	22	48	
GROUP 2	44.47	9.951	2.413	39.35	49.59	28	67	<0.001
GROUP 3	44.53	9.07	2.2	39.87	49.19	35	69	
Total	40.11	11.689	1.723	36.64	43.58	22	69	

Table 2: Age distribution in Group I, Group II and Group III

	GROUP		
Age	GROUP 1	GROUP 2	GROUP 3
21 - 30	9	1	0
31 - 40	2	5	7
41 - 50	1	8	7
> 50	0	3	3
Total	12	17	17

Table 3: Gender distribution in Group I, Group II and Group III

Gender	GROUP1	(%)	GROUP 2	(%)	GROUP 3	(%)
MALE	2	17%	10	59%	9	53%
FEMALE	10	83%	7	41%	8	47%
Total	12		17		17	

Table 4: Comparison of clinical parameters between Group I, Group II and Group III at baseline and post 6 weeks

Clinical Parameters	GROUP I Baseline	GROUP II		P VALUE	GROUP III		P VALUE
		Baseline (Mean ± SD)	Post treatment (Mean ± SD)		Baseline (Mean ± SD	Post treatment (Mean ± SD)	
PI	0.367± 0.208	1.618 ± 0.322	0.79± 0.446	P<0.05*	1.512 ± 0.359	0.663± 0.286	P<0.05*
PD (mm)	1.628± 0.609	3.663± 0.328	2.786± 0.337	P<0.05*	3.762± 0.422	2.669± 0.675	P<0.05*
CAL(mm)	0.125± 0.088	2.682± 0.825	2.014± 1.071	P<0.05*	1.597± 0.642	1.0165± 0.552	P<0.05*
mSBI (%)	7.911± 5.866	70.854± 24.246	39.303± 22.113	P<0.05*	63.784± 13.873	33.917± 14.437	P<0.05*
SOD (U/L)	184.83± 135.71	602.51± 74.258	507.01± 92.928	P<0.05*	628.31± 83.268	194.02± 96.594	P<0.05*

Table 5: Mean reduction in the clinical parameters from baseline to 6 weeks in Group II and Group III (study group)

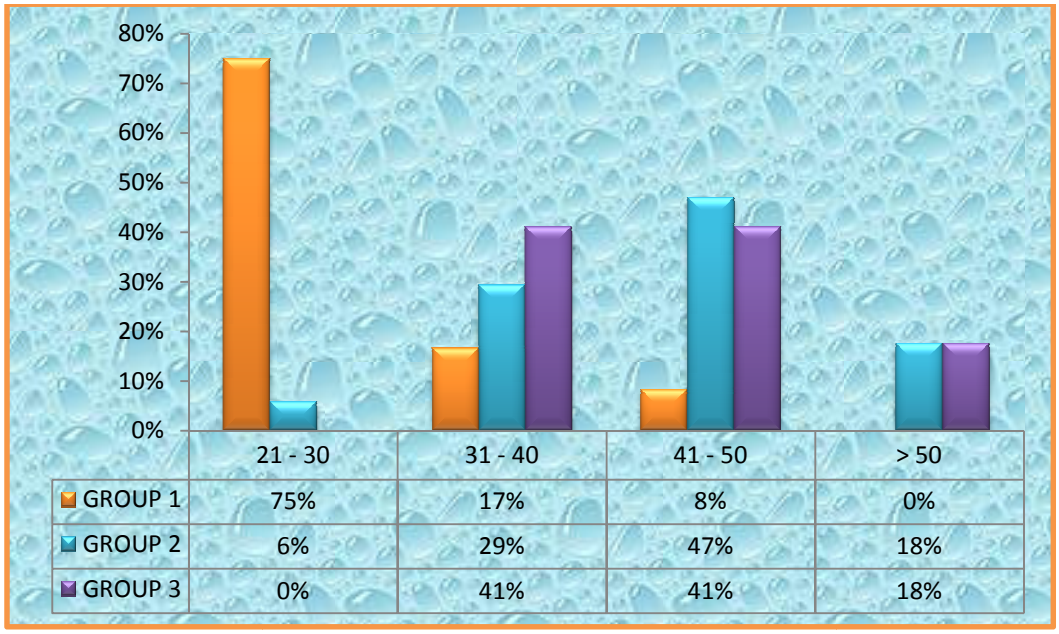
Groups	Mean Reduction in PI ± SD	Mean Reduction in PD ± SD (mm)	Mean Reduction in CAL ± SD (mm)	Mean Reduction in mSBI ± SD (%)	Mean Reduction in SOD ± SD (U/L)
GROUP II	0.8282± 0.48823	0.8771± 0.3364	0.6682± 0.4429	31.552± 21.164	95.5± 74.028
GROUP III	0.8488± 0.3858	1.093± 0.469	0.58± 0.4194	29.867± 14.199	434.28± 100.309*

Note: * denotes significance of (P<0.05).

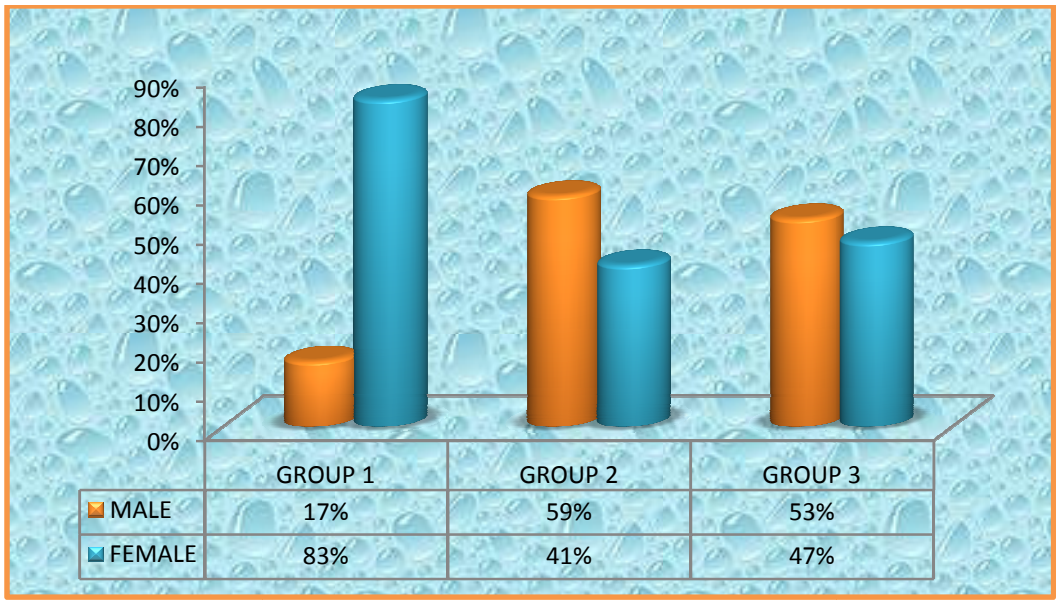
Table 6: Intergroup Difference of The Percentage Reduction In The Respective Parameters Between 6 Weeks To Baseline Using Mann Whitney U Test

GROUPS		Percentage reduction in PI	Percentage reduction in PD	Percentage reduction in CAL	Percentage reduction in mSBI	Percentage reduction in SOD
GROUP II	Mean	51.18	23.943	24.929	44.529	15.85
	N	17	17	17	17	17
	SD	38.088	2.673	29.846	8.798	25.144
GROUP III	Mean	56.129	29.055	36.329	46.825	69.12
	N	17	17	17	17	17
	SD	20.419	59.679	14.072	4.058	16.004
Difference between GROUP III and GROUP II	Mean	2.487	24.604	13.2	5.341	78.003
	N	17	17	17	17	17
	SD	20.966	39.44	5.323	32.907	35.502

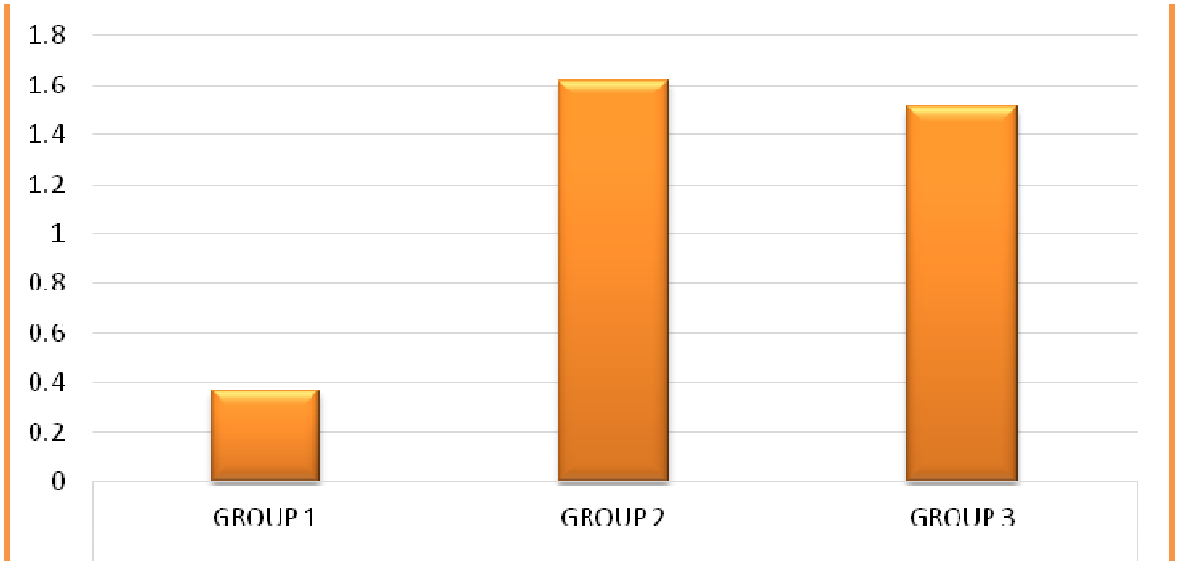
Graph. 1: Most frequenting age groups in Group I, Group II, Group III



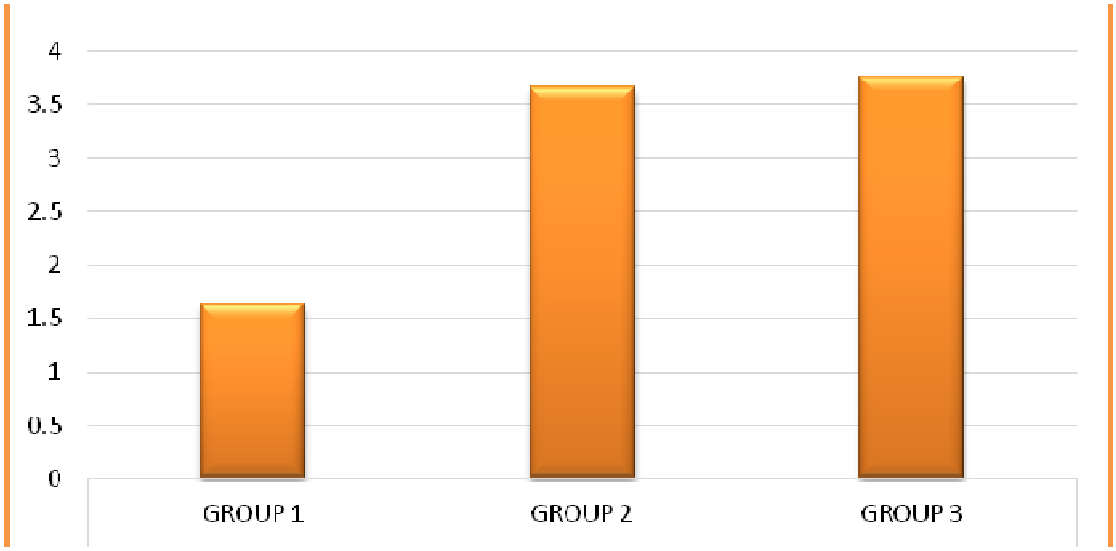
Graph. 2 : Most frequenting gender groups in Group I, Group II, Group III



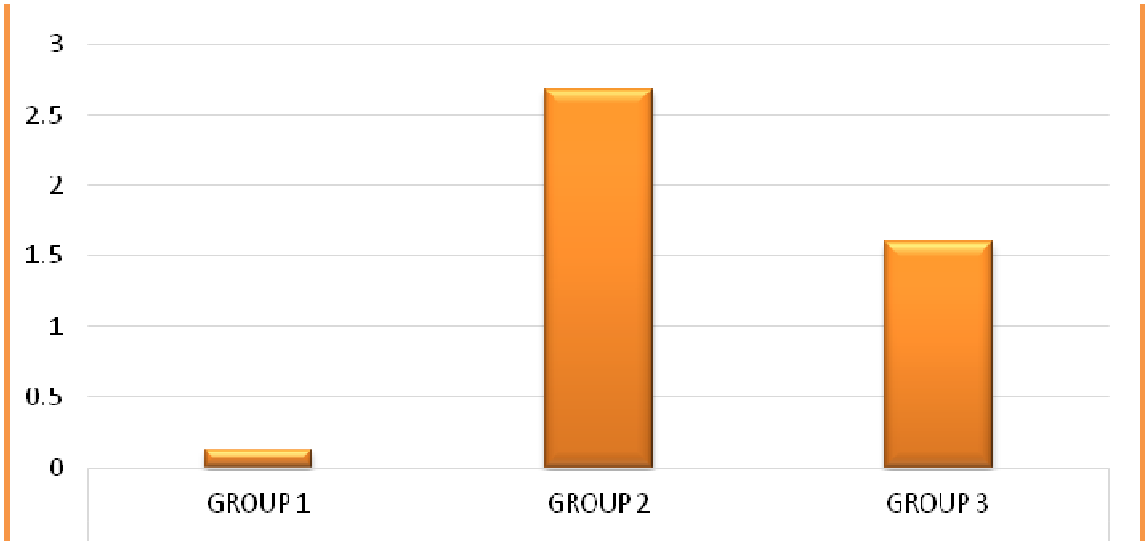
Graph. 3: Baseline-PI



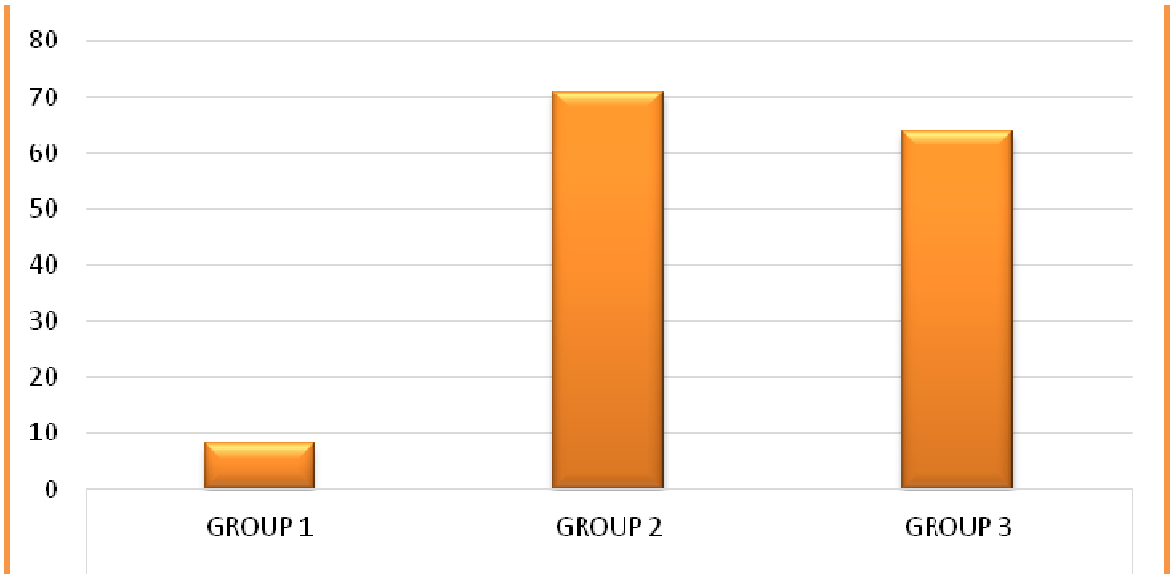
Graph. 4: Baseline-PD



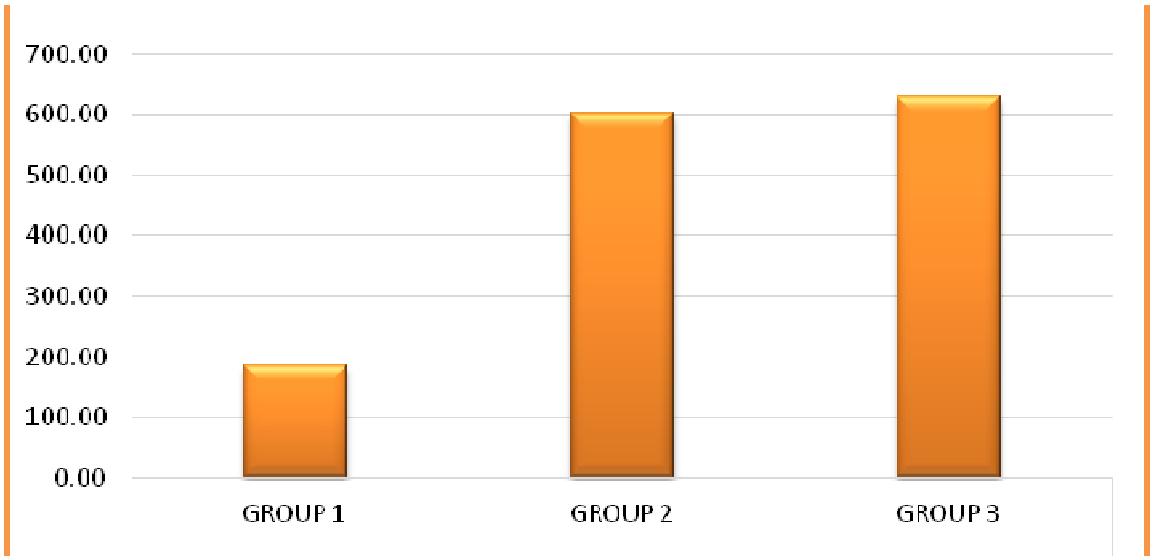
Graph. 5: Baseline-CAL



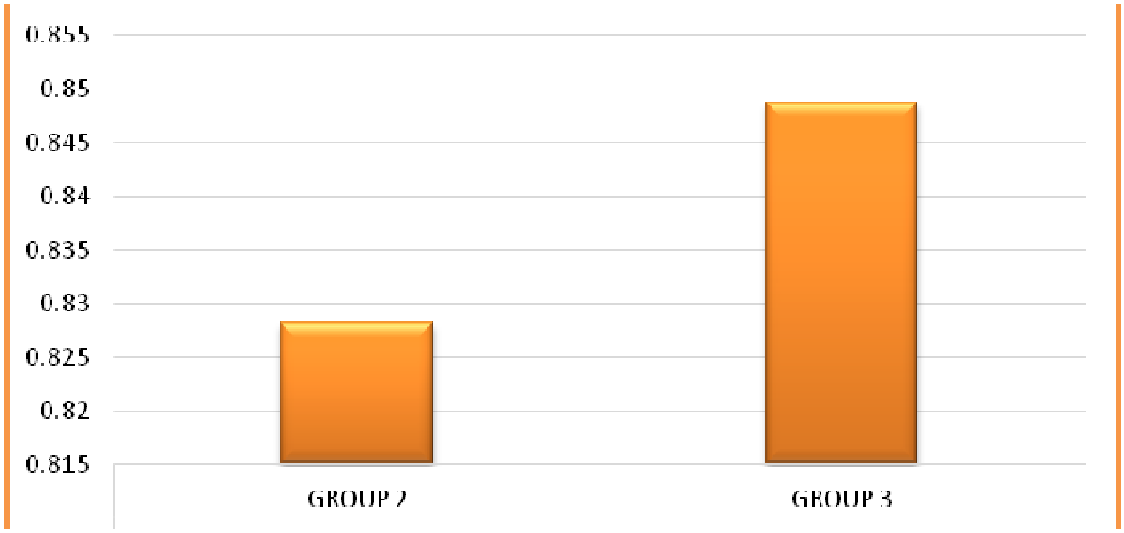
Graph. 6: Baseline- mSBI



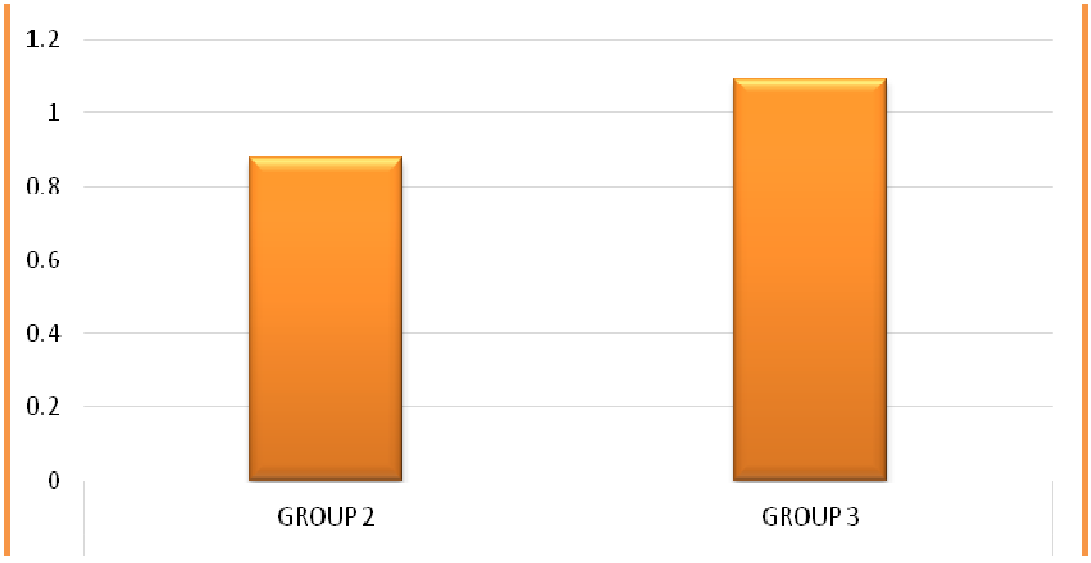
Graph. 7: Baseline-SOD



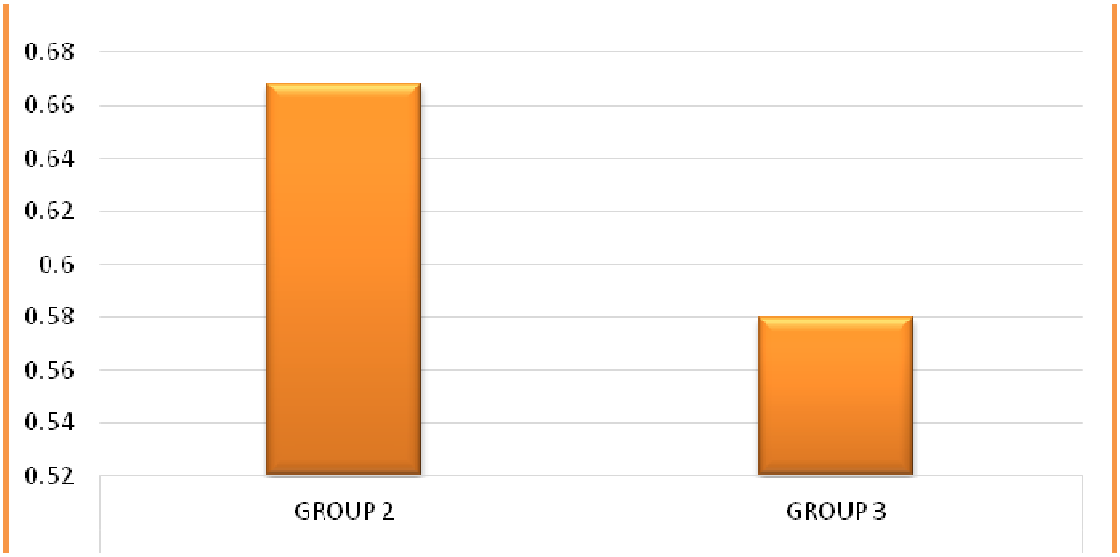
Graph.8: Mean reduction in PI



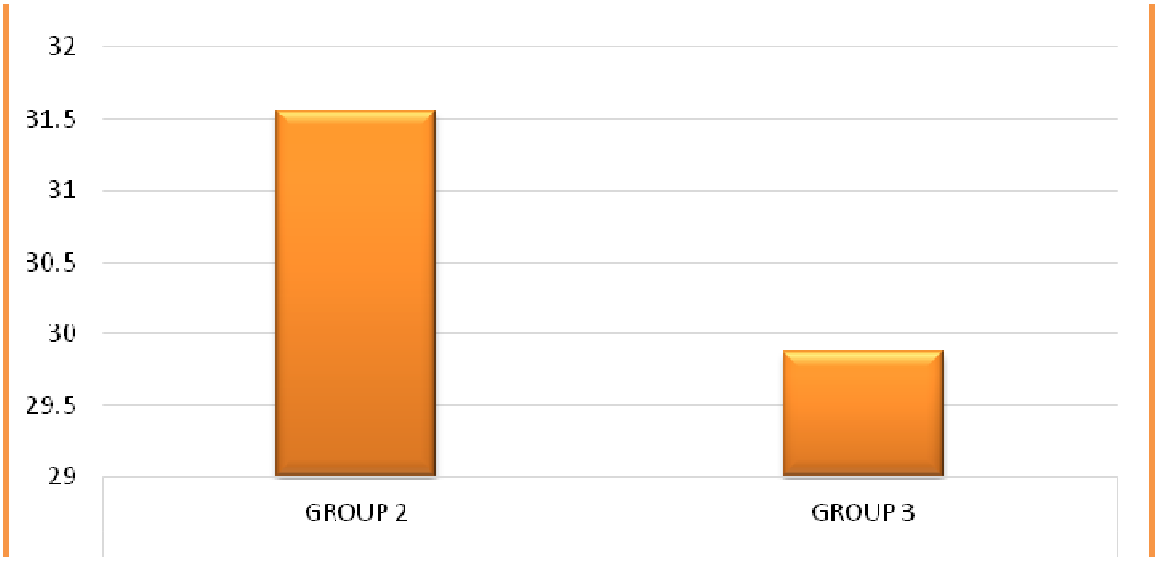
Graph.9: Mean reduction in PD



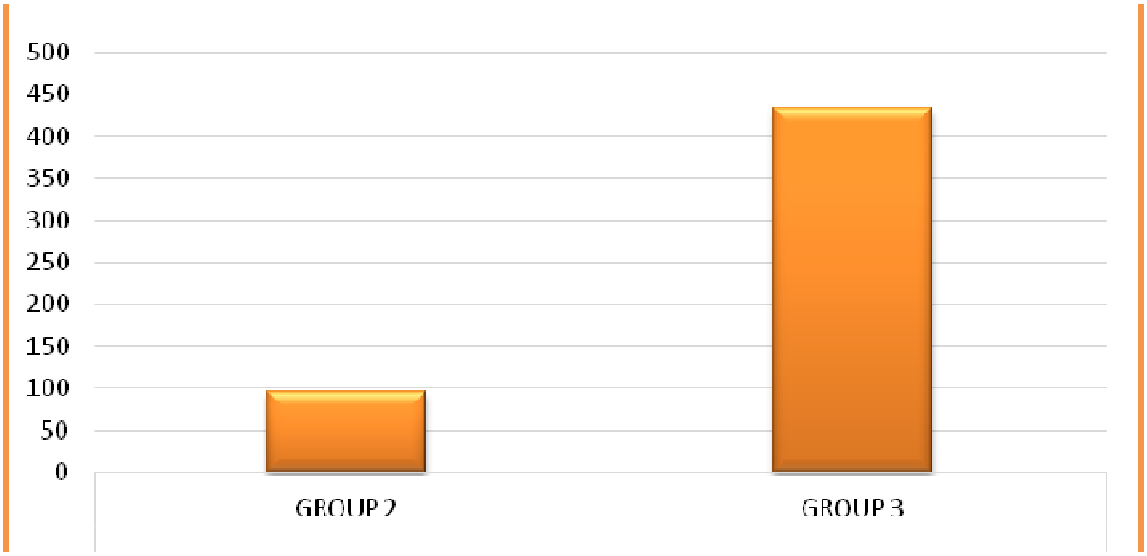
Graph.10:Mean reduction in CAL



Graph.11:Mean reduction in mSBI



Graph.12: Mean reduction in SOD



DISCUSSION

The present study had focussed on the relationship between periodontitis and the levels of SOD following the effect of SRP, with and without Vitamin -E supplementation in CP patients with respect to healthy controls.

It was suggested that patients with periodontal disease were more susceptible to oxidative stress phenomenon.[43,53] Tissue invasion by polymorphonuclear leukocytes in reciprocation to dental plaque, causes phagocytosis, resulting in excessive production of free radicals, which in turn causes damage to the cell membrane and organelles.[20] However, to combat this phenomenon, various intracellular antioxidant enzymes are naturally synthesized in the body like SOD and GPx that protects the cells, against ROS.[75] Prospective studies have associated periodontitis with Total antioxidant capacity (TAOC). TAOC reflects the full spectrum of antioxidant activity against various ROS and RNS.[76,77,78] SOD and its extracellular counterparts protects the cells against ROS by scavenging superoxide radicals and hydrogen peroxide.[79]

The action of SOD can be amplified in the presence of a lipophilic antioxidant such as Vitamin E. Vitamin E forms the first line of defense against the peroxidation of cell membrane polyunsaturated fatty acid. A positive correlation between lipid peroxidation and Vitamin E levels was reported in various cases of chronic inflammatory diseases. [80,81]

With this background, a clinical study was conducted to assess the changes in the GCF levels of SOD using ELISA at baseline and 6 weeks post SRP in CP patients with and without Vitamin E supplementation with respect to healthy controls.

The systemically healthy individuals with clinically healthy periodontium were taken as controls and the clinical parameters and SOD levels in GCF were assessed at baseline. In CP patients, the clinical parameters and SOD levels in GCF were assessed at baseline and 6 weeks following intervention and with and without Vitamin E supplementation.

In this study, the demographic characteristics include the age and gender distribution. The mean age of the subjects enrolled in Group I, Group II and Group III were 27.67±8.172 years, 44.47±9.951 years and 44.53±9.07 years respectively.

The gender distribution noted amongst healthy controls was 17% males and 83% females. Group II patients comprised of 59% males and 41% females. Group III patients comprised of 53% males and 47% females.

At baseline, the mean PI scores was significantly higher in Group II patients ($p<0.05$) which was followed by Group III and Group I in the descending order. Patients in Group II and Group III showed reduction in plaque scores of 0.8282± 0.4882 and 0.8488±0.3858 respectively, by the end of 6 weeks following SRP, which was not statistically significant. The results generated in the present study was in accordance with the results obtained in various studies. **Akpinar et al. 2013,**[57]conducted an interventional study, in which the mean reduction in PI scores was 1 in CP patients, from baseline to 6 weeks post therapy, which was statistically significant ($p<0.05$). **Sukhtankar et al 2013,**[56]conducted an interventional study, in which the results showed an improvement of 1.59 in the PI scores from baseline to 8 weeks post SRP in CP patients, which was statistically significant ($p<0.05$). **Singh et al. 2013,**[68] conducted a study in which the mean reduction in PI scores was 1.41 from

baseline to 12 weeks post SRP in CP patients, following Vitamin –E supplementation,which was statistically significant ($p<0.05$).

At baseline, the mean PD was significantly higher in Group III patients ($p<0.05$) which was followed by Group II and Group I in the descending order. Patients in Group II and Group III showed reduction in probing depths of 0.8771 ± 0.3364mm and 1.093 ± 0.469mm 0.87respectively, by the end of 6 weeks following SRP, which was not statistically significant. The results generated in the present study was in accordance with the results obtained in various studies. **Kim et al. 2010,**[54] conducted an interventional study, in which the mean reduction in PD was 0.4±05 mm, which was statistically significant ($p<0.05$). In a study by **Akpinar et al. 2013,**[57] it was found that, the mean reduction in PD was 1mm at moderate pocket sites from baseline to 6 weeks post therapy, which was statistically significant ($p<0.05$). **Sukhtankar et al 2013,**[56] concluded that there was an improvement in the PD from baseline to 8 weeks post SRP with the mean reduction being 1mm, which was statistically significant ($p<0.05$). **Singh et al. 2013,**[68]conducted a study in which the mean reduction in PD was 1.68mm from baseline to 12 weeks post SRP in CP patients, following Vitamin E supplementation, which was statistically significant ($p<0.05$).

At baseline, the mean CAL scores was significantly higher in Group III patients ($p<0.05$) which was followed by Group II and Group I in the descending order. Patients in Group II and Group III showed gain in CAL with scores of 0.6682± 0.4429mm and 0.58±0.4194mm respectively, by the end of 6 weeks following SRP, which was not statistically significant. Various other studies had similar results as in

the present study. **Kim et al. 2010,**[54] demonstrated in an interventional study, in which the gain in CAL was 0.5 mm from baseline to 4 weeks post SRP, in CP patients, which was statistically significant ($p<0.05$). **Singh et al. 2013,**[68] reported a study in which the mean gain in CAL was 1.76mm from baseline to 12 weeks post SRP in CP patients, following Vitamin E supplementation, which was statistically significant ($p<0.05$).

At baseline, the mean mSBI was higher in Group III patients ($p<0.05$) followed by Group II and Group I in the descending order. Patients in Group II and Group III showed reduction in the bleeding scores of $31.552 \pm 21.164\%$and $29.867 \pm 14.199\%$ respectively, by the end of 6 weeks following SRP, which was not statistically significant. Similar results were obtained in the following studies. **Kim et al. 2010,**[54]conducted an interventional study, in which improvement in the bleeding scores from baseline to 4 week post therapy was 37.7% ($p<0.05$), which was statistically significant. **Singh et al. 2013,**[68] performed a study in which the mean reduction in the bleeding scores was 60.76% following Vitamin-E supplementation, from baseline to 12 weeks post SRP in CP patients, which was statistically significant ($p<0.001$).

The mean GCF SOD levels at baseline was significantly high in Group III ($p < 0.05$) (628.31 ± 83.268 U/L) (P<0.05) (628.31followed by Group II (602.51 ± 74.258U/L) (602.51and healthy controls (Group I) (184.83 ± 135.71U/L), (184.83that further reduced to 194.02 ± 96.594 U/L in Group III and to 507.01 ± 92.928 U/L in Group II after 6 weeks following SRP. Patients in Group II and Group III showed a reduction in the levels of SOD by 95.5 ± 74.028 U/L and 434.29 ± 100.309 U/L respectively by the end of 6 weeks following SRP ($p<0.05$).

The results generated in Group II was in accordance with the results obtained in the following studies. **Wei et al. 2010,**[53]showed a significant reduction in the GCF levels of SOD from baseline to 16 weeks post SRP in CP patients in which the reduction was$134 \pm 3.43\text{U/mg protein}$ $(p < 0.05)$, which was statistically significant. **Kim et al. 2010,**[54]conducted an interventional study to compare the SOD activity in the saliva of CP patients before and after SRP. The results of the study showed a significant improvement of 9.6% ($p<0.05$), in the mean salivary SOD activity following SRP at the end of 4 weeks, which was statistically significant. **Sukhtankar et al. 2013,**[56]compared the SOD status in CP patients in gingival tissue samples before and after SRP at baseline and 8 weeks post therapy. The results of the study showed a significant improvement in the mean SOD activity of$184 \pm 0.87\%$ $(p < 0.001)$, which was statistically significant. **Aziz et al. 2013,**[43]compared the SOD activity in serum in subjects with CP before and after SRP from baseline to 12 weeks. The results of the study showed a significant improvement in the serum SOD activity of 84.8U/g Hb ($p<0.001$), following SRP, which was statistically significant.

There are also contradicting studies to the results obtained in our study, though those studies were assessed in saliva and serum. The mean levels of SOD derived in Group III was in contrast with the results obtained in the study performed by **Singh et al. 2013,**[68] in which there was an increase in the SOD levels of over 14.36% in the salivary and over 85.9% in the serum components, following SRP from baseline to 12 weeks, with and without Vitamin -E supplementation ($p<0.05$). **Karim et al. 2012,**[55] conducted an interventional study that had assessed the salivary and GCF-SOD levels following SRP in CP patients from baseline to 15 days post therapy. The results of the study showed an improvement of over 100.8% in the salivary and over 53.6% GCF-

SOD levels from baseline to 15 days post therapy ($p<0.005$). This could be attributed to the fact that in chronic periodontal diseases, reduction in the SOD levels was due to the inactivation of hydrogen peroxide formed, that resulted in lowering the action of SOD at the diseased site, whereas an in increase in the SOD levels in normalcy was observed due to the constant dismutation of the singlet oxygen. However, the results obtained in our study was in accordance with the studies conducted by **Wei et al. 2010,[53] Kim et al. 2010,[54] Sukhtankar et al. 2013[56]** and **Aziz et al. 2013**.[43] According to these studies, it was proved that the SOD activity increases with the progression of infection and inflammation in CP patients. This can be understood by analysing the fact that, human periodontal ligament exhibits SOD that provides biological protection against ROS, primarily singlet oxygen, during the inflammatory process.[31] Increased SOD levels were observed in periodontal diseases due to the excessive production of O_2^- by PMN's at the diseased site leading to oxidative stress. In addition to the above it was also believed that bacterial LPS was known to stimulate O_2^- release from the gingival fibroblast, featuring the production of SOD which may represent an important defence mechanism of the fibroblast during inflammation.[82,83,84,89] The SOD levels decreases after intervention due to the decrease in inflammation and bacterial LPS levels.

Therefore, in this study, there was significant improvement in clinical parameters and the levels of SOD in GCF had reduced significantly in Group III when compared to Group II ($p<0.05$). Improvement in the antioxidant status was seen by the end of 6 weeks post-operatively which was due to the reduction in the inflammation and oxidative stress. There was commendable reduction in the SOD values for patients who were supplemented with Vitamin E when compared to those

who were not supplemented with Vitamin E. So this study has shown that with the supplementation of Vitamin E in conjugation with SRP aids in reducing the levels of oxidative stress.[58,85,86]

The study was conducted in a single centre with the availability of very limited number of samples. In order to overcome this limitation, we need to perform multi-centred longitudinal studies with large sample sizes to substantiate these results and adequate provision need to be made to overcome this limitation.

SUMMARY & CONCLUSION

Chronic Periodontitis is an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss and bone loss.[1]

In CP, there is an enhanced liberation of ROS, resulting in oxidative stress. In order to counteract the ROS, the antioxidant mechanism synthesized in the human body is SOD, which is excreted in body fluids including the serum, GCF and saliva. In this study, the body fluid assessed is GCF as it is more site specific for periodontitis. In this study, we are supplementing Vitamin E, to enhance the action of SOD.

Hence this study was performed to assess the change in GCF levels of SOD, 6 weeks post SRP, in CP patients without and with Vitamin E supplementation in Group II and Group III respectively. The clinical parameters (PI, PD, CAL and mSBI%) and SOD levels were analysed at baseline and 6 weeks post SRP in both the groups.

The data were recorded and statistical analysis was performed using paired t-test, independent t-test and non-parametric Mann-Whitney U test. Results showed that all the clinical parameters improved after SRP in Group II and Group III. With regard to the levels of SOD in GCF, patients belonging to Group III showed a statistically significant greater reduction in the SOD levels 6 weeks post SRP when compared to Group II patients.

The following conclusions were drawn from the study,

1) The levels of SOD and the clinical parameters at baseline were higher in the test groups (Group II and Group III). Healthy controls had the least SOD levels and clinical parameters.

2) All the clinical parameters were significantly reduced in test groups (Group II and Group III), however a greater reduction was observed in Group III patients when compared to Group II patients, 6 weeks after SRP.

3) SOD levels in GCF, reduced significantly in Group III patients when compared to Group II patients, 6 weeks after SRP.

4) Overall, patients being supplemented with Vitamin E (Group III) showed better antioxidant response to SRP than those without Vitamin E supplementation (Group II).

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